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National Institute of Neurological  
Disorders and Stroke

# Intramural Research



Annual Report  
Fiscal Year 1989

U.S. DEPARTMENT  
OF HEALTH  
AND HUMAN SERVICES  
Public Health Service  
National Institutes of Health

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October 1, 1988 through September 30, 1989

Office of the Director, Division of Intramural Research

National Institute of Neurological Disorders and Stroke

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\* \* \* Requiring Chief





## ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

### DIVISION OF INTRAMURAL RESEARCH

National Institute of Neurological Disorders and Stroke  
October 1, 1988 through September 30, 1989

Irwin J. Kopin, M.D., Scientific Director

The Division of Intramural Research (DIR), National Institute of Neurological Disorders and Stroke, conducts investigations in a wide array of disciplines related to the neurosciences. The DIR comprises the Office of the Director, the Clinical Neurosciences Program and the Basic Neurosciences Program.

The Office of the Director includes the Animal Health and Care Section and the Coordinator for AIDS research. The Clinical Neurosciences Program consists of eight Branches and a Neuroimaging Section while the Basic Neurosciences Program contains eleven Laboratories and the Instrumentation and Computer Section. The Clinical Neurosciences Program is located mainly in the Clinical Center where there are both inpatient facilities and outpatient clinics, as well as supporting laboratories. The Basic Neurosciences Program is accommodated mainly in Building 36 with important components in the Park Building in Rockville and at Fort Detrick in Frederick, Maryland. Research requiring marine animals is conducted during the summer months in facilities rented from the Marine Biological Laboratory in Woods Hole, Massachusetts.

Federal Government scientists, support staff, guest researchers and volunteer workers, continue to contribute new discoveries which have significant impact on the explosive growth of knowledge about the nervous system and its disorders. Research is initiated by scientists with interests ranging from the fundamental basis for molecular interactions regulating growth, development, function, and mechanisms of drug action in the nervous system--to clinically relevant development of new diagnostic and therapeutic procedures targeted on the early diagnosis, prevention, retardation, cure, or symptomatic control of neurological disorders. The results of these investigations contribute to the advance of many aspects of biomedical knowledge, the prevention or alleviation of human suffering from disease or injury, the main mission of the Institute and the NIH. The important

accomplishments and the status of potentially major advances toward understanding neuronal function and dysfunction are summarized in the subsequent Laboratory/Branch reports and in the investigator-initiated research summaries of the FY 1989 Annual Report. This portion will address primarily issues which have a major impact on the administration and resources (personnel, available space, and financing) of the Institute.

The management of the Division of Intramural Research is a team effort which involves the active participation of Dr. Mark Hallett, Director of the Clinical Neurosciences Program (who is also Clinical Director, NINDS); Dr. Ernst Freese, Director of the Basic Neurosciences Program; and the Scientific Director. The administrative skills, scientific expertise, wise counsel of the Program Directors, along with able administrative officers, have contributed immensely to the smooth operation of the DIR by the Director.

The creation of the new National Institute of Deafness and Other Communicative Disorders (NIDCD) was accomplished by separating from NINDS those components which conducted research relevant to the mission of the new Institute. Resources formerly supporting the Laboratory of Neuro-Otolaryngology, headed by Dr. Jorgen Fex, were transferred (along with appropriate personnel) to NIDCD where the Laboratory has been renamed Laboratory of Molecular Otology. Also, transferred were the Unit on Speech and Voice Pathology (headed by Dr. Christy Ludlow) from the Medical Neurology Branch, along with the Audiology Unit and the ENT Consultation Service, both from the OCD. NINDS support services (administration, personnel) have continued to be available to the new Institute, but it is understood that this is a temporary measure which will remain in effect only until NIDCD establishes its own support services. New research findings and new initiatives in neuroscience, as well as organizational changes attendant in evolution of the staff and room allocations to NINDS, continually create needs which require personnel and other changes to utilize optimally the available resources.

### Personnel

As in previous years, the DIR has utilized fully its employment ceiling. Young investigators provide depth of expertise for future roles and expansion of research leadership in new high priority research efforts. They are particularly important because the discrepancy in salaries between government and academic institutions

or industry makes it extremely difficult, if not impossible, to attract senior established investigators to NIH. At present there are over 475 employees accounting for the 467 full-time equivalent (FTE) positions. Requirements for expanded efforts on research of AIDS have necessitated some shifts in FTE positions.

The personnel of the DIR includes 135 professional non-tenured employees: 13 medical staff fellows, 26 staff fellows, 48 senior staff fellows, 11 special experts, and 37 visiting associates/visiting scientists. There are also a total of 144 FTE-ceiling exempt scientists (VF and IRTA positions; National Research Council fellows ) who are subject to a NIH -imposed ceiling, as well as guest workers, volunteers, and IPA appointments.

The evolution in the NINDS staff resulted from the retirement of Dr. William Adelman, Chief, Laboratory of Biophysics (LB) and of Dr. Henry Wagner, Chief, Cerebrovascular Pathophysiology Section in the Laboratory of Neuropathology and Neuroanatomical Sciences (LNNS). Dr. Gerald Ehrenstein is now Acting Chief, LB. Dr. Wagner, a former Director of Intramural Research, NINDS, continues to conduct research at NIH as a Health Scientist Emeritus.

After an extended search, Dr. Gustavo Roman has been recruited to fill the position of Chief, Neuroepidemiology Branch and will begin on January 1, 1990. This position has been vacant for over two years since the tragic death of Dr. Bruce Schoenberg in 1987.

Scientists continue to be attracted away from NIH, in large part, because of more advantageous financial arrangements (higher salary, college tuition for children, less restriction on consultation to industry, etc.). Dr. Donald Gehlert, a tenure track Senior Staff Fellow in Experimental Therapeutics Branch has accepted an offer in private industry. The relatively low salary scales at NIH have frustrated attempts to appoint a Chief for the Laboratory of Neuronal Growth and Regeneration (LNGR). The Laboratory has been disestablished and Dr. Zalewski, who had been Acting Chief, has been reassigned to the Laboratory of Neural Control (LNC) as Chief, Neural Regeneration Section. Other components which would have been part of LNGR have been included in the Surgical Neurology Branch. Dr. Robert Burke, Chief, LNLN, has established a new Section of Developmental Neurobiology headed by Dr. Michael O'Donovan. The Laboratory of Neurochemistry, under the leadership of Dr.

Harold Gainer, has established a new Molecular Neuroscience Section under Dr. James Battey who transferred from the NCI.

We are continuing efforts to recruit an established junior investigator to form a core neurogenetics group. The individual selected is expected to have a strong developmental neurobiology background. So far, potential candidates have accepted other positions.

Dr. Alison Wichman has elected to join the Clinical Center, Bioethics Office; Dr. Barbara Karp has been appointed to replace her as Chief of the Neurology Consultation Service.

During the last year, six NINDS scientists have been approved for tenure or intent-for-tenure positions. Dr. Jordon Grafman (formerly Senior Staff Fellow) will be head of a Unit on Cognitive Neuropsychology in the Medical Neurology Branch (MNB). Dr. Susan Chang will oversee the technical aspects of the new Central Electron Microscopy facility for the NINDS (established in the Laboratory of Neurobiology) . Dr. Michael Rogawski, who will be Chief of the Unit on Neuronal Excitability in the MNB; Dr. Jeffery Alger, formerly a Special Expert in our Neuroimaging Section, will be responsible for the NINDS component of the experimental MRI facility; Dr. Anne Schaffner has been appointed a collaborative investigator in the Laboratory of Neurophysiology and Dr. Leonardo Cohen, Visiting Scientist in the MNB, has been approved for intent-for-tenure.

### Space

Inadequacy of space on the NIH campus requires that some of the NINDS scientists work in rented facilities in the Park Building. Even upon completion of Building 49 this situation will persist and a search for additional off-campus laboratory space is in progress. The maintenance of a critical mass to ensure scientific interactions and avoidance of feelings of isolation are important considerations in any off-campus facility. At present, most of an entire large laboratory (Laboratory of Molecular and Cellular Neurobiology, LMCN) is housed in the Park Building; a portion of Dr. Daniel Alkon's Section on Neural Systems is accommodated in Building 9. The investigators in LMCN will be given space in Building 49 when available, but a portion of LMCN may still have to be housed off-campus. Included in this Laboratory is the NINDS Central DNA Sequencing Facility under the supervision of Dr. Craig Venter, Chief of

the Section for Receptor Biochemistry and Molecular Biology. This facility assists NINDS laboratories in preparing and sequencing oligonucleotides relevant to neuroscience research.

Animal facilities to meet AALAC accreditation standards continues to be the ultimate goal of the Animal Health and Care Sections of the NIH. All Institute animals in Building 36 (except primates with immunodeficient virus infections, primates in LNLC and a small colony of HSV-infected mice) were moved into the combined animal facility on the mezzanine level of Building 36. This new facility provides superb holding, procedural space, and excellent environmental controls.

Building 10A should be available to house all animals in Building 10 and negotiations with Space Management for compensation of loss of NINDS space in Building 10A are near satisfactory completion. NINDS will receive about 15 modules, all but two of which have been identified. Space will be made available for Dr. Dalakas to pursue his studies on muscular disorders and AIDS.

Building 14D is being completed and the scientists of the Surgical Neurology Branch who are now working Building 9 may be able to move there before the end of Fiscal Year 1990.

Problems of patient admissions have begun to plague the clinical programs because the "swing space" for ward renovations has been allocated to the NIH efforts in AIDS research. Current negotiations with the Clinical Center and other Institutes may help to resolve these problems.

### Fiscal Issues

The current level of support is adequate to continue the important research operations of the Institute, but additional funds may be required if requests for additional personnel are allocated to expand high priority research work on AIDS, tissue implants and gene therapy, and any additional off-campus space needs to be rented. Decisions regarding efforts in electromagnetic imaging and magnetic resonance spectroscopy await responses to requests for additional funding.



### Cooperation With Industry

Scientists in NINDS will be initiating, or already have initiated, Cooperative Research and Development Agreements, or CRADAs, with industrial organizations with a goal of commercializing products developed within their laboratories. Three such CRADAs, negotiated in 1989 include: (a) cloning NMDA and dopamine receptors, and investigate neural expression of muscarinic acetylcholine receptor subtypes (Dr. Brann, LMB); (b) testing the efficacy of HIV vaccine in chimpanzees (Dr. Gibbs, LCNSS); and (c) developing techniques for large scale DNA sequencing (Dr. Venter, LMCN).







# ANNUAL REPORT

October 1, 1988 through September 30, 1989

Office of the Clinical Director, OD, DIR

Office of Director, Clinical Neuroscience Program, DIR

National Institute of Neurological Disorders and Stroke

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Office of the Director

Office of the Clinical Director

Clinical Neuroscience Program, DIR

Mark Hallett, M.D., Clinical Director

The Office of the Clinical Director handles administrative matters, mainly relating to patient care, coordination of educational activities, and delivery of neurological services. Service functions can be divided into the EEG Laboratory, the EMG Laboratory, the Consultation Service for Neurology, Neuromuscular Services, Neuropathology, and Paraprofessional Support Services. The Otolaryngology Consultation Service and the Audiology Laboratory moved during the year to the National Institute of Deafness and Other Communicative Disorders.

The two major educational conferences are the Clinical Conference (held on Tuesday afternoon), which is aimed at the Medical Staff Fellows and typically reviews a patient in detail, and the NINDS Grand Rounds (held on Friday afternoon). The Clinical Conference includes some attention to matters of patient care and quality assurance. The Grand Rounds continues to offer CME credit.

**EEG Laboratory, Susumu Sato, M.D., Chief**

Diagnostic Services:

The total number of tests performed during this reporting period showed a slight increase due to a significant increase in EEG testing, although a moderate decrease was noted in evoked potential testing. During this reporting period we experienced some changes in the laboratory personnel.

|                 | EEG | Evoked Potentials |
|-----------------|-----|-------------------|
| NINDS           | 158 | 160               |
| NINDS OPD       | 275 | 32                |
| NIADDK          | 9   |                   |
| NICHD           | 95  | 55                |
| NIMH            | 120 | 2                 |
| NCI             | 83  |                   |
| NHLB            | 18  | 2                 |
| NIAID           | 9   | 6                 |
| NIA             | 95  | 1                 |
| CCM, SICU & CSR | 28  | 7*                |
| OTHER OPD       | 86  |                   |
| TOTAL           | 976 | 265               |

\*intraoperative evoked potential recording

## Participation in Research Activity:

The EEG Laboratory continues to work closely with the Clinical Epilepsy Section of the Medical Neurology Branch and plays an important role in evaluating epileptic patients. In this respect, the laboratory staff monitor and interpret EEG recording during pentylentetrazol intravenous injection, sodium amyltal intracarotid injection, surgery for treatment of epilepsy (electrocorticography) and during chronic subdural recording in epileptic patients. The laboratory staff assist in applying electrodes for magnetoencephalographic study and neuropsychological investigation.

The collaboration has been made to monitor EEG from deeply anesthetized and irradiated monkeys and do the same monitoring in patients, including those with Lowe's syndrome.

The EEG Laboratory provides a training environment for a Medical Staff Fellow toward certification by the American EEG Board. The Laboratory Chief continues to serve as Associate Examiner on the EEG Board.

### EMG Laboratory, Roger W. Gilliatt, M.D., Chief

#### EMG ACTIVITIES (July 1, 1988-June 30, 1989)

|                        |                  |     |
|------------------------|------------------|-----|
| Number of Examinations | NINDS            | 203 |
|                        | OTHER INSTITUTES | 209 |
|                        | Total            | 412 |

Approximately half of the referrals to the EMG Laboratory during the year originated within NINDS, and the other half were requested from other Institutes. Part of the work of the Laboratory consists of routine diagnostic and prognostic studies of patients under the care of the Clinical Center; the other part consists of special studies on agreed projects. These may be in collaboration with other Institutes or may be initiated from within the laboratory.

- 1) Studies on AIDS and other HIV positive patients (collaborative studies with the National Cancer Institute)

The main role of the laboratory has been to monitor HIV positive patients, including AIDS and ARC patients, for signs of neuropathy during treatment with experimental drug regimes (AZT/DDC/DDI combinations.)

- 2) Studies on patients treated with suramin for malignant disease (collaborative studies with the National Cancer Institute)

Since the unexpected finding made in 1987 that some patients with high blood levels of suramin may develop severe life-threatening polyneuropathy, we have monitored a large numbers of patients to ensure that early signs of toxicity are detected, and dose regimes modified accordingly.

- 3) Patients with polymyositis (collaborative program with the National Institute of Arthritis and Musculoskeletal and Skin Diseases.)

These patients are referred for the special treatment of inflammatory muscle disease with steroids, immunosuppression or plasmapheresis. We are using a technique of quantitative electromyography originally described by Willison (1964), which involves analysis of the interference pattern of the EMG during pattern of the EMG during sustained muscle contractions against a standard load. There have been problems with this technique in the past which have seemed to limit its usefulness but with certain modifications, we believe it is providing an accurate indication of the severity and extent of underlying inflammatory muscle disease.

- 4) Evaluation of neuromuscular disease (NINDS Study #84-N-203; Principal Investigator, Dr. Mark Hallett) include:
- a. A comparison of proximal and distal nerve conduction in Charcot-Marie-Tooth (CMT) disease.
  - b. The refractory period of transmission (RPT) in human entrapment lesions.
  - c. Sensory conduction in diabetic neuropathy.

#### Publications:

Gilliat R, Meer J. The refractory period of transmission in the carpal tunnel syndrome. *Muscle Nerve* 1988;11:974.

Meer J, La Rocca R, Dalakas M, Gilliat R, Pezeshkpour G. Demyelinating neuropathy with conduction block following suramin therapy for malignant disease. *Neurology* 1989;39:294.

Nilsson J, Ravits J, Hallett M. Stimulus artifact compensation using biphasic stimulation. *Muscle Nerve* 1988;11:597-602.

#### **Consultation Service, Barbara Illowsky Karp, M.D., Chief**

The Neurology Consultation Service consists of three neurologists: Dr. Barbara Karp (Chief), Dr. Eric Wasserman, and Dr. Alison Wichman. The service provides emergency and routine consultations to patients hospitalized in the Clinical Center and to outpatients referred to the Ambulatory Care Research Facility. In 1988, a total of 589 were seen in this service. Outpatients are referred to the bi-weekly Neurology Clinic or evaluated in other specialty clinics (Oncology, Hematology, Surgery). As a part of the Consultation Service, we facilitate the performance of necessary diagnostic procedures in other departments (i.e., myelography, electromyography, head scans), and arrange neurological follow-up as indicated.

## Neuromuscular Services, Marinos C. Dalakas, M.D.

Dr. Marinos Dalakas provides clinical neuromuscular evaluations and expert advice in the diagnosis and management of patients with neuromuscular symptoms referred to us by all the Institutes. He has established and directs the Laboratory of Muscle Enzyme Histochemistry to provide a state-of-the-art histochemical and immunocytochemical evaluation of muscle and nerve biopsies. He performs the muscle and nerve biopsies in the operating room using local anesthesia and the biopsied specimens are processed for a battery of histochemical reactions. Approximately 100 biopsies per year are performed and processed in his laboratory. The findings are presented to clinical conferences of the respective Institutes or in informal teaching sessions that Dr. Dalakas holds for the Fellows and other interested investigators.

The Laboratory of Muscle Enzyme Histochemistry also processes and reviews several muscle biopsy specimens sent to us from outside hospitals for expert advice. The referring physicians are subsequently called and the management of a diagnosed neuromuscular disorder is discussed. If a disorder fits within one of our protocols, patients are brought in for further study. Some of the collaborative investigative studies Dr. Dalakas is currently actively involved in include studies of patients with: a) polymyositis/dermatomyositis (Dr. Plotz) NIMSD, b) chronic fatigue syndrome due to EBV/CMV infection (Dr. S. Strauss) NIAID, c) Nephropathic cystinosis and muscle carnitine deficiency (Dr. W. Gahl) NICHD, d) Duchennes' muscular dystrophy (Dr. Podolsky) NIMSD, and e) acquired immunodeficiency syndrome (AIDS) (Dr. Yarchoan) NCI and f) Neurological complications from suramin therapy.

In addition to the above neuromuscular services and collaborations with other Institutes, Dr. Dalakas is performing independent research in neuromuscular disorders under approved clinical protocols, studying patients with: a) post-polio syndrome, b) immune polyneuropathies, c) motor neuron disease, and d) neuromuscular aspects of human immunodeficiency virus infection.

Dr. Dalakas has recently assumed the responsibility of the clinical coordinator for the AIDS activities in the NINDS, and Chief of the newly formed Neuro-AIDS Unit to conduct clinical and basic studies in the neurological aspects of HIV infection.

In his new capacity, he has organized a Unit to investigate the neuromuscular aspects of HIV infection and involvement of the neuromuscular system by several retroviruses, including HIV-1, HTLV-1 and simian retroviruses I and III. In addition, he has been instrumental in establishing a clinical protocol via an Interagency Agreement with the Naval Medical Hospital to investigate longitudinally the neurological, virological and immunological manifestations of newly seroconverted HIV-positive patients during a 3 year period.

### Publications:

Willison HJ, Trapp BD, Bacher JD, Dalakas MC, Griffin JW, Quarles RH. Demyelination induced by intraneural injection of human anti-myelin associated glycoprotein antibodies. *Muscle Nerve* 1988;11:1169-76.

Yarchoan R, Thomas R, Fischel MA, Grafman J, Wichman A, Dalakas MC, Jacobier FK, Broder S. Treatment of human immunodeficiency virus-associated neurological disease with 3'-Azido-2',3'-dideoxythymidine. In: Bolognesi D, ed. *Human retroviruses, cancer and AIDS: approaches to prevention and therapy*. New York: Alan Liss, 1988:393-406.



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Dalakas MC. Post-polio syndrome. In: Yearbook of Nursing 88. Springhouse, PA: Springhouse, 1988;50-4.

Dalakas MC, Hallett M. The post-polio syndrome. In: Plun, F. ed. Advances in contemporary neurology. Philadelphia, FA Davis, 1988;51-94.

Dalakas MC. Pathogenesis of post-polio syndrome: Clues from muscle and CNS studies. In: Munsat T, ed. The post-polio syndrome. Butterworths 1989, in press.

Madden DL, Mundon FK, Tzan NR, Fuccillo DA, Dalakas MC, Calabrese V, Elizan TS, Roman GC, Sever JL. Serological studies of patients with multiple sclerosis, and patients with other neurological diseases: antibodies to HTLV-I, HTLV-II, HIV, and STLV-III. In: Cazzullo CL, et al, eds. Virology and immunology in multiple sclerosis: rationale for therapy. Germany: Springer-Verlag, 1988;43-50.

Su YL, Ho K, Dalakas M, Mutchnick MG. Localization of immunoreactive thymosin  $\alpha 1$  in astrocytes of normal human brain. *Ann Neurol*, 1989, in press.

Dalakas M. Advances in the neurological investigation of the late effects of polio. In: "Proceedings of National Conference on the late effects of Polio", Ontario March of Dimes, Toronto, Canada, 1988;13-34.

#### **Neuropathology, David A. Katz, M.D.**

As in previous years, diagnostic Neuropathology Service for NINDS, and for all other Institutes, have been provided by Dr. Katz. The Neuropathology Service is integrated with the Autopsy, Surgical Pathology, and Ultrastructural Pathology Sections and residency training program of the Laboratory of Pathology, NCI; a high priority is given to resident teaching. The brain was examined in a high percentage of autopsies performed at NIH in the last year. Many manifested significant primary or secondary neurological disease, including malignant gliomas, dementias, neurological complications

of systemic malignancy, and AIDS, particularly in the pediatric age group. Braincutting, held weekly, is scheduled so as to encourage participation by interested physicians and nurses. Relevant neuropathological findings are presented formally at gross autopsy conference and mortality conferences. Selected cases are further utilized for neurological clinical conferences. Neurosurgical specimens include both in-house and submitted materials, for an annual total of approximately 250 cases; intra-operative frozen-section consultations are required in approximately 50 in-house cases per year (surgical material includes: primary and metastatic brain tumors, spinal tumors and vascular lesions, and brain biopsies [AIDS, PML], and pituitary tumors).

The Neuropathology Service functions in a collaborative manner to provide subspecialty expertise in a range of clinicopathologic investigations. Continuing active collaborations include: (1) participation in dementia and degenerative disease protocols from NIMH (Trey Sunderland, M.D.), NIA (Stanley Rapoport, M.D.), and NINDS (Experimental Therapeutics Branch and Movement Disorders Clinic); (2) study of patients with progressive multifocal leukoencephalopathy (PML) (Sidney Houff, M.D., Ph.D.); (3) correlation of pathological and neuroimaging data in temporal lobectomy specimens from epileptic patients (William Theodore, M.D.); and (4) participation in experimental treatment protocols for malignant gliomas with both Surgical Neurology Branch, NINDS, and Radiation Oncology Branch, NCI. Additional work in progress include studies of cis-platinum toxicity (Eddie Reed, M.D., NCI), familial leukodystrophy (Roswell Eldridge, M. D., NINDS), and of cerebral 'atrophy' in pediatric AIDS patients (Stanley Rapoport, NIA).

#### **Paraprofessional Support Services**

Linda Nee, MSW, is assigned to the Clinical Neuropharmacology Section, Clinical Neuroscience Branch. She has been pursuing clinical and family studies, organizing field clinics and undertaking genetic counselling.

Helen Krebs, RN, assigned to the Neuroimmunology Branch, is taking a major role in running a clinical trial of the use of cyclosporin in multiple sclerosis.

Marjorie Gillespie, RN, is assigned to the Experimental Therapeutics Branch; she supports several aspects of the clinical program.



and Radiation Oncology Branch, NCI. Additional work in progress include studies of cis-platinum toxicity (Eddie Reed, M.D., NCI), familial leukodystrophy (Roswell Eldridge, M. D., NINDS), and of cerebral 'atrophy' in pediatric AIDS patients (Stanley Rapoport, NIA).

### **Paraprofessional Support Services**

Linda Nee, MSW, is assigned to the Clinical Neuropharmacology Section, Clinical Neuroscience Branch. She has been pursuing clinical and family studies, organizing field clinics and undertaking genetic counselling.

Helen Krebs, RN, assigned to the Neuroimmunology Branch, she is taking a major role in running a clinical trial of the use of cyclosporin in multiple sclerosis.

Marjorie Gillespie, RN, is assigned to the Experimental Therapeutics Branch; she supports several aspects of the clinical program.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ODIR  
ZO1 NS 02675-05

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Neuromuscular Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                      |     |      |     |       |
|---------|----------------------|----------------------|-----|------|-----|-------|
| PI:     | Mark Hallett, M.D.   | Clinical Director    | OCD | ODIR | DIR | NINDS |
| Others: |                      |                      |     |      |     |       |
|         | Roger Gilliatt, M.D. | Chief, EMG Lab       | OCD | ODIR | DIR | NINDS |
|         | Jacob Meer, M.D.     | Medical Staff Fellow | OCD | ODIR | DIR | NINDS |
|         | Louis Johnson        | EMG Technician       | OCD | ODIR | DIR | NINDS |

## COOPERATING UNITS (if any)

National Cancer Institute

## LAB/BRANCH

Office of the Clinical Director, Office of the Director, CNP, Division of Intramural Research

## SECTION

EMG Laboratory

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1

## PROFESSIONAL:

0.9

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Understanding of neuromuscular diseases is founded on careful clinical observation, electrodiagnostic studies and pathology. This protocol has been carried out to learn more about established diseases, to characterize new diseases, to assess current methodologies and technologies, and to refine old methods and develop new ones.

Studies on acquired immune deficiency (AIDS) and other HIV positive patients (collaborative studies with the National Cancer Institute). The main role of the laboratory has been to monitor HIV positive patients, including AIDS and ARC patients, for signs of neuropathy during treatment with experimental drug regimes (AZT/DDC/DDI combinations).

Studies on patients treated with suramin for malignant disease in collaborative studies with the National Cancer Institute. Since the unexpected finding made in 1987 that some patients with high blood levels of suramin may develop severe life-threatening polyneuropathy, we have monitored large numbers of patients to ensure that early signs of toxicity are detected, and dose regimes modified accordingly.





ANNUAL REPORT  
October 1, 1988 through September 30, 1989

Neuroimaging Section, OCD, CNP, DIR  
National Institute of Neurological Disorders  
and Stroke

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ANNUAL REPORT  
October 1, 1988 through September 30, 1989  
Neuroimaging Section, OCD, CNP, DIR  
National Institute of Neurological  
Disorders and Stroke

Giovanni Di Chiro, M.D., Chief

SUMMARY

Honors, Awards

"Cum Laude": 74th Annual Meeting of the Radiological Society of North America, November 27-December 2, 1988, Chicago. Scientific Exhibit: "MR Imaging of Cerebrospinal Fluid Spaces and the Neuraxis: Flow and Motion Relationships."

"Summa Cum Laude": 27th Annual Meeting, American Society of Neuroradiology, March 19-24, 1989, Orlando, Florida. Scientific Exhibit: "MR Imaging of Cerebrospinal Fluid Spaces and the Neuraxis: Flow and Motion Relationships."

"Best paper": Southern Neurosurgical Society Annual Meeting, March 9-12, 1989, Point Clear, Alabama. "Positron emission tomography in the detection of malignant degeneration of low-grade gliomas."

Dr. Di Chiro was made "Honorary Member of the European Society of Neuroradiology." (Paris, France).

Dr. Di Chiro delivered the "Torgny Greitz Lecture" at the Karolinska University (Stockholm, Sweden).

Dr. Di Chiro was asked to participate in the video history (video-tape) of developments in neurosurgery and related sciences. This history is prepared for the archives of the "American Association of Neurological Surgeons" (AANS, formerly Cushing Society).

Research Summary

Following is a summary of the major findings for the research protocols of the Neuroimaging Section in the fiscal year October 1, 1988 through September 30, 1989.

Radiographic and Radioisotopic Angiography of the Spinal Cord.  
(Project #Z01 NS 01195-25) Angiographic studies of arteriovenous malformations and vascular tumors of the spinal cord have continued. Recent observations indicate that magnetic resonance imaging (MRI), particularly in combination with the paramagnetic contrast agent Gd-DTPA, is of higher value than previously estimated in the diagnostic assessment of these lesions. Also, on the basis of three observations, it has been possible to confirm that a myelopathic syndrome may originate from an intracranial dural arteriovenous malformation draining into spinal veins. Finally, we have initiated a feasibility study of MR

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angiography which, if successful, will allow us to assess the vascular pathological lesions of the spinal cord noninvasively.

Nuclear Magnetic Resonance (Imaging and Spectroscopy) and Computed Tomography (Transmission). (Project #Z01 NS 02073-16)

The NMR imaging research has developed along several lines in both imaging and spectroscopy:

Continuing the MRI assessment of patients with spinal cord pathology, particularly arteriovenous malformations (AVMs). The MRI diagnostic yield with these lesions is continuously improving. We have been able to confirm, by repeat MRI studies, that cord cavities associated with hemangioblastomas will decrease in size (or disappear) after surgical removal of the tumoral nodule(s). Recently we have initiated work with MR angiography (flow based studies) in cord AVMs and cine-MRI in patients with syringomyelia and other cord cavities.

Analysis of artifactual linear regions of abnormal signal intensity along the length of the spinal cord. These MRI artifacts, which are related to sampling (Gibbs phenomenon), have been confused with cord pathology (syringomyelia, arteriovenous malformations).

Studies of pulsatile CSF flow using longitudinal imaging (flow direction in the image plane) and phase reconstruction. This new method is advantageous for its speed and simplicity; pulsatile flow direction and velocity may be easily assessed, and reliable information about CSF bulk flow is obtained.

Recognition by phase imaging of the normal spinal cord pulsation-motility, and its disappearance in cases of "fixed" cord (due to tethering, scarring, tumor compression).

Imaging the spinal epidural veins. We are particularly interested in flow within these vessels and its modifications (Valsalva's maneuver, intraspinal tumors, intervertebral disc herniations).

Imaging the pars compacta of the substantia nigra in normal subjects and in patients affected by Parkinson's disease (hemiparkinsonian subjects are of particular interest).

Analysis of the signal intensity changes in MRI of patients affected by a variety of movement disorders (iron accumulation?).

Continuing in vivo and in vitro investigation of the relaxation times ( $T_1, T_2$ ) of extravasated intracranial blood. Changes in  $T_1$  and  $T_2$  are, in great part, related to the chemical modifications and progressive denaturation of hemoglobin.

Trying to learn more about the NMR signal of various abnormal tissues. Particular attention has been devoted to the signal from CNS tumors of various types and grades. We are also engaged in a comparative "in vivo/in vitro" study of  $T_1$  and  $T_2$  of normal and pathological CNS tissues.



Comparing clinical MRI imaging results with those of CT and particularly PET in a variety of abnormal conditions, with emphasis on CNS tumors.

Imaging with experimental, small bore (animal studies), 2 Tesla and 4.7 Tesla imaging-spectroscopy NMR devices.

Imaging (2T) of PML affected (experimental model) monkeys: Our goal is the early recognition of demyelinating processes.

Imaging of monkeys of various ages at 0.5, 1.5, 2.0 and 4.7 Tesla to assess the iron content in the basal ganglia, its increase with age and the possible modifications in pathological conditions (experimental parkinsonism). Iron distribution is verified by Perls' stain of brain specimens.

Successful demonstration by MRI of selective basal ganglia damage following intracarotid injection of MPTP.

NMR spectroscopic (P-31, H-1) studies (4.7 T) of experimentally induced cerebral ischemia in gerbils.

Editing of proton spectra to obtain lactate signals free of fat contributions (2.0 and 4.7 T).

Probably our most important contribution this year is represented by the successful initiation of proton spectroscopy at 1.5 Tesla in human patients. For the moment we have concentrated our attention on brain tumors (some 15 patients studied already).

CT studies of conditions such as degenerative diseases of the CNS, cavities of the brain stem and spinal cord, and particularly brain and spinal cord tumors, have continued.

Positron Emission Tomography (Project #Z01 02315-12) A follow-up FDG-PET study has been completed of a series of patients initially harboring low grade gliomas which later changed grade. The FDG-PET method has been found optimal for monitoring the grade change (malignant degeneration).

Confirmation has been obtained that the FDG-PET method is the optimal procedure for differentiating tumor recurrence from post radiation and/or postchemotherapy cerebral necrosis.

The FDG-PET method of tumor evaluation has been extended from the gliomas to other intracranial tumors, particularly meningiomas. We have clear indications that FDG-PET is an excellent method to predict "post-removal" recurrence of meningiomas.

We have initiated a study with the FDG-PET method of pituitary microadenomas. From preliminary observations, it appears that the method allows the recognition of some of these small lesions.

A study of F-18 tagged 6-fluorodopa has been done in normal monkeys, monkeys made parkinsonian (and particularly hemi-parkinsonian) with the neurotoxin MPTP, as well as parkinsonian monkeys treated with intracerebral grafting (adrenal medullary and fetal mesencephalic) and simple cavitation of the caudate head. 6-fluorodopa imaging of the basal ganglia in these three groups of monkeys has proven to be a useful and reliable means to assess the state of dopaminergic innervation (correlation with clinical status) and to evaluate function and destiny of the transplants. We have also offered PET evidence of catecholaminergic fiber sprouting.

Finally, 6-fluorodopa PET scanning has been used in seven parkinsonian patients treated with adrenal medullary autografts. We are in the process of analyzing these studies.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01195-25 OCD

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Radiographic, Radioisotopic, CT, and MR Angiography of the Spinal Cord\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Giovanni Di Chiro, M.D., Chief, Neuroimaging Section, OCD, CNP, DIR, NINDS  
Others:

|                      |       |           |
|----------------------|-------|-----------|
| J. L. Doppman, M.D.  | Chief | DRD, CC   |
| E. H. Oldfield, M.D. | Chief | SN, NINDS |
| R. D. Neumann, M.D.  | Chief | NMD, CC   |

COOPERATING UNITS (if any) Diagnostic Radiology and Nuclear Medicine Departments,  
Clinical Center, NIH; Surgical Neurology Branch, NINDS; Medical  
Examiner's Office, Department of Public Health Philadelphia, PA.

## LAB/BRANCH

Office of Clinical Director, CNP, DIR

## SECTION

Neuroimaging

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Selective arteriography (radiographic) of the spinal cord is a diagnostic technique which has proven to be informative in cases of arteriovenous malformation (AVM), tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers some advantages as a screening method, and in certain types of intraspinal pathology may give information not available by any other diagnostic test.

Experience with the techniques of dynamic computed tomography, digital subtraction angiography (DSA), positron emission tomography (PET) using  $^{18}\text{F}$ -2-deoxyglucose and nuclear magnetic resonance imaging (MRI) of the spine (without and with a contrast agent such as Gd-DTPA) indicates that these methods may be useful screening and follow-up procedures in the evaluation of certain vascular lesions and tumors of the spinal cord.

\*Formerly: "Radiographic and Radioisotopic Angiography of Spinal Cord"

|  |                      |                                       |
|--|----------------------|---------------------------------------|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                      | PROJECT NUMBER<br>Z01 NS 02073-16 OCD |
| PERIOD COVERED<br>October 1, 1988 through September 30, 1989   |                      |                                       |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)<br>Nuclear Magnetic Resonance and Computed Tomography (Transmission)   |                      |                                       |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>Giovanni Di Chiro, M.D., Chief, NIS, OCD, CNP, DIR, NINDS<br>Others (NIS): J. R. Alger, Ph.D., Special Expert; R. A. Brooks, Ph.D., Staff Physicist; L. M. Levy, M.D., Ph.D., Guest Researcher; A. Bizzi, M.D., Visiting Fellow; B. DeSouza, M.D., Staff Fellow; R.S. Miletich, M.D., Ph.D., Staff Fellow; M.J. Fulham, M.D., Special Volunteer<br>J.L. Doppman, M.D., Chief, DRD, CC<br>Joanna M. Hill, M.D., Senior Staff Fellow, NSB, NIMH   |                      |                                       |
| COOPERATING UNITS (if any)<br>Diagnostic Radiology, Clinical Center, NIH; "In Vivo" NMR Research Center, BEIB, NIH; NSB, NIMH; Division of Neuropathology of Case Western Reserve University Medical School, Cleveland.  |                      |                                       |
| LAB/BRANCH<br>Office of Clinical Director, CNP, DIR  |                      |                                       |
| SECTION<br>Neuroimaging  |                      |                                       |
| INSTITUTE AND LOCATION<br>NINDS, NIH, Bethesda, MD 20892   |                      |                                       |
| TOTAL MAN-YEARS:<br>2.0  | PROFESSIONAL:<br>2.0 | OTHER:                                |
| CHECK APPROPRIATE BOX(ES)<br><input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither<br><input checked="" type="checkbox"/> (a1) Minors<br><input type="checkbox"/> (a2) Interviews   |                      |                                       |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)<br>NMR: Our NMR imaging research is developing along eleven main lines: 1) study of patients with spinal cord pathology, particularly arteriovenous malformations (AVM); 2) recognition of artifactual linear regions of abnormal signal intensity along the length of the spinal cord; 3) assessment of pulsatile CSF flow using longitudinal imaging; 4) studies of the "mobile" (normal) and "fixed" (pathologic) spinal cord; 5) continuing the <u>in vivo</u> and <u>in vitro</u> investigation of extravasated intracranial blood; 6) analysis of the signal intensity from critical areas (basal ganglia) in patients affected by a variety of movement disorders; 7) trying to learn more about the NMR signal of various abnormal tissues; 8) comparing clinical MRI imaging results with those of CT and particularly PET; 9) Analysis of iron accumulation in the basal ganglia of normal primates of various ages as well as in parkinsonian (MPTP) animals; 10) MRI of MPTP induced lesions in the basal ganglia of primates; 11) animal CNS imaging and spectroscopy with experimental, small bore devices (2.0 and 4.7 Tesla). Finally, quite recently, we have initiated A) investigation of NMR spectroscopy (proton) in patients with brain tumors; B) cine-MRI applications to the assessment of patients with syringomyelia and other cord cavities; C) MR angiography (flow based) in cord AVMs; and D) comparison of diffusion (Intra-Voxel Incoherent Motion Analysis) with PET in brain tumors.<br><br>CT: Ongoing research studies of tumoral, degenerative, demyelinating and atrophic processes of the brain, plus hydrocephalus, brain edema, post-radiation necrosis and epilepsy. |                      |                                       |
| 6 OCD/DIR(NIS)   |                      |                                       |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02315-12 OCD

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Positron Emission Tomography

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|  |                              |
|--|------------------------------|
| Giovanni Di Chiro, M.D., Chief, NIS, OCD, CNP, DIR, NINDS. | OTHERS:                      |
| R. A. Brooks, Ph.D.  | Staff Physicist NIS, NINDS   |
| R. S. Miletich, M.D., Ph.D.                                | Sr. Staff Fellow NIS, NINDS  |
| M. J. Fulham, M.D.   | Special Volunteer NIS, NINDS |
| G. Comi, M.D.  | Visiting Fellow NIS, NINDS   |
| B. DeSouza, M.D.   | Staff Fellow NIS, NINDS      |
| I. J. Kopin, M.D.  | Director DIR, NINDS          |
| E. H. Oldfield, M.D.                                       | Chief SN, NINDS**            |

## COOPERATING UNITS (if any)

NM, CC, SN, NINDS; MN, NINDS; DIR, NINDS; BEIB, DRS; DRD, CC; LCM, NIMH;  
OCD, NINDS, CNB, NINDS

## LAB/BRANCH

Office of Clinical Director, CNP, DIR

## SECTION

Neuroimaging

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

3.0

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Positron Emission Tomography (PET) allows us to obtain anatomical data (e.g., axial transverse or coronal images of the brain) as well as dynamic functional data, such as regional cerebral glucose consumption rate, using  $^{18}\text{F}$ -2-deoxyglucose (FDG). Besides FDG, other radiopharmaceuticals (tagged with  $^{18}\text{F}$ ,  $^{15}\text{O}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$ ) can be used with PET to study the BBB, oxygen metabolism, protein synthesis, and neuroreceptors. The unique property of PET is that it provides physiologic and pathophysiologic information not available with any other imaging procedure.

-----  
\*\*Continued:

|                      |                   |            |
|----------------------|-------------------|------------|
| C. V. Kufta, M.D.    | Staff Physician   | SN, NINDS  |
| M. Hallett, M.D.     | Clinical Director | DIR, NINDS |
| R. J. Polinsky, M.D. | Staff Physician   | MN, NINDS  |
| L. Sokoloff, M.D.    | Chief             | LCM, NIMH  |

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Publications:

Alger JR, Brunetti A, Nagashima G, Hossmann KA. Evaluation of a newly-discovered water suppression pulse sequence for high field in vivo H-1 surface coil NMR spectroscopy. Magn Reson Med 1989;11:73-84.

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Hatazawa J, Brooks RA, Dalakas MC, Mansi L, Di Chiro G. Cortical motor-sensory hypometabolism in amyotrophic lateral sclerosis: A PET study. J Comput Assist Tomogr 1988;12:630-6.

Levy LM, Di Chiro G, McCullough DC, Dwyer AA, Johnson DL, Yang SL. Fixed spinal cord: diagnosis with MR imaging. Radiology 1988;169:773-8.

vanZijl PCM, Moonen CTW, Alger JR, Cohen JS, Chesnick SA: High field localized proton spectroscopy in small volumes: greatly improved localization and shimming using shielded strong gradients. Magn Reson Med 1989;10:256-65.

Wrobel CJ, Doppman JL, Di Chiro G, Oldfield EH. Myelopathy due to intracranial dural arteriovenous fistulas draining intrathecally into spinal medullary veins. J Neurosurg 1988;69:934-9.

IN PRESS

1. Alger JR, Brunetti A. Phase distortion-free in vivo surface coil proton spectroscopy. Magn Reson Med.
2. Brooks RA, Brunetti A, Alger JR, Di Chiro G. On the origin of paramagnetic inhomogeneity effects in blood. Magn Reson Med.
3. Dubinsky RM, Hallett M, Levey R, Di Chiro G. Regional brain glucose metabolism in neuroacanthocytosis. Neurology.
4. Dubinsky RM, Greenberg M, Di Chiro G, Baker, Hallett M. Hemiballismus. In: Movement disorders.
5. Di Chiro G. Form and function in the neuroradiological image: A personal overview. Proceedings of 1983 meeting: "Da Luigi Galvani alla Moderna Neurobiologia", Bologna University, IX Centennial Meeting.









# ANNUAL REPORT

October 1, 1988 - September 30, 1989

## Instrumentation and Computer Section

Office of Director, Basic Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

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## INSTRUMENTATION & COMPUTER SECTION

Office of Director, Basic Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

October 1, 1988 - September 30, 1989

Bruce M. Smith, Ph.D., Chief

The Section on Instrumentation and Computers (ICS) provides technical support for investigators of NIMH and NINDS, Divisions of Intramural Research (DIR's) by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing; and (4) managing a central computer facility consisting of a multiuser MicroVAX 3600, an image processing system, and a network of Macintosh personal computers and LaserWriter printers.

Additional services provided by the Section include consultation on measurement techniques, signal processing, mathematical and statistical analysis techniques, and equipment and computer purchases. Formal or informal instruction for individual investigators or groups are offered by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS will take on the project. If a commercial product will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Chief agrees to accept a project, the investigator submits a standard ICS work request form, signed by his Lab Chief. This form states the nature of the instrument or service requested, and should contain as many details and specifications as the investigator can provide. The project is then assigned to an engineer or computer staff member who confers with the investigator to formulate a complete set of specifications and a cost estimate for the project. ICS does not charge for services, however, upon completion of the project, the investigator's laboratory or branch is billed for the cost of the components used. Reimbursement of funds takes place at the beginning of the next fiscal year.

## INSTRUMENTATION

The Section has a staff of four engineers, five computer specialists and five technicians to design and produce special-purpose instrumentation. The availability of powerful, low-cost personal computers and single-chip microprocessors has broadened the Section's approach to instrumentation development. It is often appropriate for an engineer, a technician, and a computer specialist to work together to combine electronic or mechanical hardware, a personal computer or microprocessor, and custom software, to produce cost-effective solutions to complex instrumentation problems. The following are brief descriptions of the Section's major projects, taken from a total of 329 projects undertaken this year.

Ambulatory Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the hardware and software which forms the system. Monitor: The current version of the PAM has a 1K-byte memory and is in its seventh year of production. Approximately 125 monitors are in use, with the Section providing battery changes and repairs as needed. The development of an improved monitor with a 32K-byte memory and one-minute activity intervals was begun last year. Significant revisions to that design have been made this year which add flexibility and should increase data integrity. Computer Support: The Section supports a PAM readout system based on the Macintosh personal computer coupled to a microprocessor-controlled serial interface. A comprehensive PAM program has been written for the Macintosh to handle data readout and disk filing, graphical data editing, construction of continuous data files and raster plots, and formation of tabular data sets for transfer into spreadsheet and statistical applications. Twelve of the PAM interfaces for the Macintosh have been fabricated for IRP studies; an additional five units have been supplied in support of collaborative efforts outside of the IRP's. Development has begun on revisions of the Macintosh readout program and the serial interface to accommodate the new PAM's increased memory capacity and to take advantage of its much higher readout speed. IMS CRADA: In May, 1989 the NIMH and Individual Monitoring Systems, Inc. (IMS) entered into a Cooperative Research and Development Agreement (CRADA) in order to work together to further the development of the PAM technology for the benefit of the NIMH and the general public. With a non-exclusive license to the NIMH PAM patent, IMS is developing the current version of the monitor for commercial sales to research and medical markets. IMS and the Section plan to utilize their joint expertise to finalize the new PAM design, to develop a new readout program and serial interface, and to pursue the extension of the PAM technology to monitor additional parameters.

Light Monitoring System. The behavior of animals during research activities may be influenced by variations in the circadian light cycles which they receive in their cage environments. A monitoring system has been developed to allow the managers of the NIMH animal rooms to accurately document these light/dark cycles. The system consists of three units: (1) a battery-powered light monitor for each room that senses and stores the condition of the room light during each minute interval for up to 22 days; (2) a battery-powered portable reader which is used to initialize each light monitor and to read out and display the data; and (3) a battery-charging unit for the reader. Monitors have been installed in the eleven animal rooms in Bldgs. 9 and 10 and an additional five units will be installed in the ACRF rooms when renovations are completed.

**Drosophila Activity Monitor.** Monitoring the activity levels of *Drosophila* is essential to studies of the effects of genetics on the response to certain anesthetics. This project involved automating the quantification of the activity levels of *Drosophila* in a test tube during selected time intervals. Movement activity is detected in a specially-designed test tube chamber by a X-Y pair of infrared emitters and photodiode detectors. The detector outputs are summed and compared to an "activity count" threshold set by the investigator. Activity counts are accumulated for time intervals of 0.25, 0.5, or 1.0 minute by a single-chip microprocessor. The processor shows the current interval number and the activity count on a LCD display and at the end of each time interval, generates a hard copy record on a small thermal printer.

**Sleep Deprivation Monitor.** Sleep deprivation is a technique currently being investigated to treat patients suffering from depression and manic-depressive symptoms. Unfortunately, the therapeutic effect may be cancelled if the wakefulness is interrupted even briefly. A pocket-sized monitoring and alerting system is being developed that senses eye blinks, as an indication of wakefulness, by measuring eye muscle movement with a piezo-film transducer. As long as the eye blinks at least once in each 20-second period, the patient is considered awake. Otherwise, a piercing alarm is sounded to restore the patient to wakefulness. Following more testing, the monitor will be expanded to include internal memory to store the alarm occurrences for later readout and analysis.

**EEG Amplifier System.** An improved 32-channel EEG amplifier system is being developed for use in ongoing research projects, including topographic brain mapping. Both the preamplifier and main amplifier units have been redesigned for increased flexibility and improved signal-to-noise ratio. The new design will also allow two older systems to be updated. Each amplifier channel consists of a preamplifier, amplifier, and a selectable anti-aliasing filter. The system provides control over signal bandwidth, and provides outputs for recording by a tape recorder and 16-channel polygraph, and for digitizing and analysis of the EEG signals by a computer.

**Voice Activated Switch.** A voice activated switch was developed for use in testing vocal response times in normal and schizophrenic subjects to a battery of tasks generated by an IBM PC. The circuit processes a microphone's output signal with an adjustable-gain amplifier, a full-wave rectifier, a peak detector and a comparator switch. An LED VU display meter allows the gain to be easily adjusted to match the voice level of the subject being tested. The comparator's output changes are recorded by the computer via a game port input.

**ECG/EMG/Theta Filter Bank.** A 12-channel active filter network was fabricated for waveform monitoring in sleep research. Each channel provided separate inputs and outputs for three different types of Butterworth filters: a 4-pole lowpass/highpass combination for EEG signals (0.5 to 20 Hz); a 4-pole lowpass/highpass combination for EMG signals (5-40 Hz); and a 2-pole high-Q bandpass centered on 7 Hz for Theta signals.

**Interface for Liquid and Pellet Dispensers.** An interface has been developed which allows IBM compatible PCs to provide reinforcement during visual recognition tasks via control of liquid or pellet dispensers. Fourteen of these popular units were fabricated this year.



Computer Interface Panel. A new panel was developed to provide access to the A/D, D/A, and the clock of a PDP-11/73 computer used for psychological testing experiments. The interface allows computer control of several pieces of testing equipment, including a light stimulus/response device and an X-Y display system, and provides a system of flexible I/O connections for system expansion.

## MACHINE SHOP FACILITY

The Section maintains a well-equipped machine shop which is specialized for working with plastics and other synthetic materials, and metals. This facility is critical to the development and fabrication of the electronic and electromechanical instrumentation projects described above. Additionally, three of the Section's five technicians utilize this facility to independently specify, design, and fabricate a wide range of mechanical devices as part of the Section's efforts to provide a spectrum of services in support of basic and clinical research. These staff members are also readily available to advise investigators on mechanical principles and techniques and on the properties and uses of materials. Many investigators and other intramural staff frequently come to the shop for immediate help with a small mechanical problem whose timely solution is crucial to their ongoing research. Trained technicians from other labs use our facility to augment the limited capabilities in their own areas.

The following list illustrates the range of mechanical design and fabrication projects typically provided by our machine shop staff: a wide variety of chambers for biological preparations, including tissue cultures, electrophoresis gels, and static and dynamic temperature-controlled perfusion systems; modifications to micromanipulators and to microscopes and other optical devices; modifications to animal chairs, restraining devices and enclosures; pipette holders and storage racks, including radiation shields and collectors; a variety of Faraday cages and enclosures; human and primate holders and adapters for brain scanners; and numerous other adapters for commercial instrumentation.

## COMPUTER SUPPORT

In addition to the development of special instrumentation systems, ICS provides support for individual laboratory computer systems and maintains central computer facilities for high-capacity data storage, complex off-line data analysis, image processing, scientific word processing, and high-quality printing and plotting. These support services are detailed under the following categories.

## LABORATORY COMPUTERS

Small minicomputers and personal computers are widely used in the IRP laboratories for: real-time data acquisition and control, mathematical and statistical data analysis, graphics, and word processing. ICS provides consultation on the specification and selection of these systems and helps



the scientist in the procurement, installation and maintenance of the equipment. Training in operating systems, programming languages and maintenance issues is available for scientists or laboratory support personnel. Manpower limitations make it difficult for ICS to provide complete programming for specific individual applications. Section personnel are always available for consultation and will aid the investigator in writing the difficult time and data dependent sections of real-time programs. Section programming efforts in support of laboratory applications are concentrated on developing and maintaining a library of routines which are specifically designed to be incorporated into investigators' programs. ICS personnel also evaluate commercial software or programs from other research facilities to determine their utility for IRP laboratory systems.

ICS has selected the Apple Macintosh family of computer systems as our standard for support of scientific applications. The Section has developed considerable experience using the Macintosh Plus to provide innovative solutions for low-speed laboratory data acquisition projects. For the acquisition of real-time data and control of laboratory devices at high speeds, the Section has begun development of utility routines and applications software for the Macintosh II. The goal is to develop a laboratory data acquisition and control system, called MacNeuros, which provides equivalent features to those on older PDP-11 systems, and which will offer extended capabilities by utilization of the advanced graphical features of the Macintosh II. Due to the size and complexity of MacNeuros, the development effort will be shared by ICS and an outside contractor. ICS is developing and writing the backbone, or high-level, portion of the system and has written an extensive Request For Proposal for the outside development of carefully specified modules tailored to be compatible with the new user interface and with the functionality of the existing system. Additional modules will be developed to allow the acquisition and analysis of data from single channel recordings, averaged evoked responses, excitatory post-synaptic potentials and single unit recordings. ICS has responsibility for evaluating responses to the RFP and for overseeing and coordinating the development of the system.

## VAX FACILITY

For the past 7 years, ICS has managed a DEC VAX-11/750 computer system for use by all NIMH and NINDS intramural investigators. We have recently replaced the 11/750 with a new DEC MicroVAX 3600 system consisting of the 3600 processor and a 622 MB RA82 disk drive, a TK70 296 MB cartridge tape drive, and a TSV05 1600 BPI tape drive. In the near future, an 8 mm helical scan tape drive will be added to allow nightly backups of all files for improved system security and reliability. Two 664 MB disk drives have also been ordered for increased storage capacity. Approximately 50 hard-wired cable connections between Bldg. 36 intramural labs and the 11/750 have been moved to two new Emulex P4000 terminal servers to provide serial communication to the 3600 over Ethernet. Users can also gain access at 1200 or 2400 baud on 4 dial-up lines. All functions offered on the 11/750 have been continued on the new system. An important new addition is AlisaTalk, a software package that provides central network file and printing services to personal computers on the RSB/ICS InterNet network. The FTP program from Process Software has also been added to allow PDP-11 laboratory computers high-speed access to the 3600 via the InterNet's Ethernet backbone. This ability to rapidly transfer large files to the 3600 has made it practical to

expand the scope of VAX data analysis; for example, programs for the analysis of flow cytometric data and current-voltage relationships have been converted from PDP-11's to the 3600.

Currently, the most popular package on the VAX is the sequence analysis software from the University of Wisconsin Genetics Computing Group. This package includes over 100 programs, extensive documentation, and complete on-line help. Version 6 was recently installed on the 3600 and provides several new programs including FASTA, TFASTA, and QuickSearch for increased speed in searching the rapidly growing sequence databases. The Section also provides the complete GenBank nucleic acid database and the NBRF protein database with quarterly updates. Other popular programs on the VAX are DataPlot, an interactive program for curve fitting and graphics, and UNITY, a System V UNIX shell with Berkeley extensions that runs on top of the native VMS operating system.

## IMAGE PROCESSING FACILITY

The Section's PDP-11 based image processing facility in Bldg. 36 has been replaced by a new system using a Macintosh II, a video camera, and lightbox. The new system has most of the capabilities of the PDP-11 system, but is smaller, more reliable, significantly less expensive, and easier to use. Central to the new system is a program called Image which is being developed by Section personnel for acquiring, enhancing, analyzing, editing, animating, and pseudocoloring images. The new system is useful for numerous applications, including densitometric analysis of autoradiographs, evaluation and quantification of CT, MRI, and PET images, receptor binding studies, analysis of electrophoretic gels, and molecular modelling. To further increase its utility, a Montage film recorder has recently been added to the system to allow the production of presentation quality 35 mm slides, polaroid prints, and overhead transparencies from the Macintosh II graphical images. Because the Macintosh II system is relatively inexpensive, as well as simple to maintain, investigators with extensive image processing requirements are easily able to duplicate the Section's facility for use in their own laboratories. In fact, ten intramural laboratories are in the process of setting up their own versions of this system.

## PERSONAL COMPUTER FACILITY

Included in the Section's central facility in Bldg. 36 are two Macintosh Plus computers, three Macintosh II computers with large screen displays, an Apple flatbed scanner, a Shiva NetModem, three LaserWriter Plus printers, and a variety of software that intramural scientists can use for statistical analysis, for communicating with DCRT's mainframes and MEDLINE, and for word processing, including creation of posters, slides, and publication-quality charts and graphs. Three of the Macintoshes are also connected to the VAX and can be used to emulate VT-100 and Tektronix 4014 or 4105 terminals. The flatbed scanner provides a means of converting hard copy graphics into Macintosh graphical files and, in conjunction with optical character recognition software, allows conversion of typed documents into files compatible with a variety of word processing applications.

## COMPUTER NETWORKS

The RSB/ICS InterNet. ICS has continued to expand its network linking Macintosh, Digital, and IBM compatible computers via the AppleTalk, DECnet, and TCP/IP protocols. The network was started within ICS in Bldg. 36, and now comprises 8 LocalTalk networks, including networks in Bldgs. 9 and 10, with two more networks to be added in Bldg. 36 in the near future. An Ethernet backbone has been added and both the Macintosh II AppleShare file server and AlisaShare file server on the MicroVAX 3600 are connected to it, providing high-speed file access to computers connected via Ethernet adapter cards, and 230K bit/sec. access for the LocalTalk networks via a Kinetics FastPath gateway. Four DEC PDP-11 computers in Bldg. 36 can now perform rapid file transfers to and from the 3600 and each other over the InterNet via TCP/IP protocols. We are using AppleShare PC to provide AppleShare file services and LaserWriter printing services to IBM compatible computers over the LocalTalk networks.

3COM Token-Ring Network. Last year the Section installed a small 3COM token-ring network in Bldg. 36 in order to develop in-house expertise in token-ring technology. This prototype network is now being configured so that it can be transferred to Bldg. 10 where it will serve as the NIMH, IRP administrative network. It will provide file sharing, electronic mail and remote network services for the Office of the Director and the Administrative Offices.

## COLLABORATIVE SUPPORT

Section specialists provide collaborative support for selected research projects within the intramural laboratories by contributing their expertise in computer application, software development, and statistical analysis and experimental design. These efforts and the resulting software developments are described below.

Morphological Classification of Cells. *Fractal Geometry:* A collaborative effort is in progress with the LNP and the LNC, NINDS and the LDN, NICHD to use fractal geometry as a mathematical basis for the quantitative classification of neural cells grown in culture. The group has now published a paper on an edge detection technique which isolates and outlines digitized cell images retaining all the resolvable neurites and surface characteristics. Three computational techniques have been found that accurately calculate the fractal index of known fractal images. These techniques have been applied to neuronal images and the results have been published in two papers in the Journal of Neuroscience Methods. A third paper using fractal analysis to study the development of glial cells is in preparation. These methods have also been reported at Neuroscience and Physiological Society meetings. *Fourier Analysis:* In collaboration with LNP and LNC, a method of analyzing cell shape has been developed using a Fourier transform of the outline of cells produced by one of the edge detecting techniques. This Fourier method is now being applied to both neural and glial cell images, where it has accurately coded the essential features of cell shape in a few Fourier coefficients. It is hoped that this can become the basis of a cell classification scheme. This work has been reported at Cell Biology meetings.



Flow Cytometry Studies. A comprehensive collaborative effort is in progress with the LNP, NINDS which involves the use of voltage-sensitive dyes and flow cytometry techniques to study the electrical and pharmacological properties of embryonic rat and chick CNS neurons, and various clonal lines. Section effort is focused on experimental design, statistical and graphical analysis of data, and the development of custom software. Experimental Design and Data Analysis: ICS is intimately involved in the design of experiments and analysis of data on specific projects on development in rat spinal cord, hippocampus and striatum, and in chick spinal cord. New methods have been developed allowing for quantification of previously qualitative results on the population responses to neural transmitters and ion channel agents. Software Development: Programs have been developed for the smoothing, integrating, overlaying and statistical analysis of distributions of optical data from the flow cytometer. Publication: ICS is involved in writing and editing papers on this work especially as it applies to methodology. Papers have been published in the Journal of Neuroscience Methods and reports made at Neuroscience Society and FASEB meetings.

Non-Linear Mathematical Models. A collaborative effort has begun with the Unit on Disassociative Disorders, LDP, NIMH to utilize techniques derived from the field of non-linear dynamics (chaos) to develop a mathematical model for the analysis and classification of non-periodic recurring behavioral and physiological phenomenon.

Statistical Consultation. A service has been instituted for consultation with emphasis on rational experimental design along with a tutorial approach to statistical methods. Consultation is usually provided on a drop-in basis but can include a more formal presentation when appropriate. This year ICS took a leading role in bringing an all-day video course in chaos theory to the NIH.

VMRB Linked Databases. A collaborative project is in progress with the Veterinary Medicine and Resources Branch, NIMH to move numerous files from various applications such as MacWrite, WriteNow, FileMaker, Microsoft Works, and OverVUE into FoxBase +/Mac and organize them into an appropriately linked system of databases. The general categories of databases will be protocols, animals, and animal users (including investigators). A series of programs is being written which will allow the user to track such things as due dates for primate shots, species required by a given protocol, protocols for which 80% of allotted animals have been ordered, animal location, animal housing (space used/available), investigator training, due date for animal users' TB tests, and protocol renewal due dates. The programs are being designed to ensure data integrity and to allow the generation of reports as needed.

Bibliographic Database. A series of FoxBase +/Mac programs is being written so that an extensive bibliographic database previously developed on a PDP-11 can be moved to a Macintosh II. These programs will provide for easy additions and editing of the database, and also for searches involving complex logical combinations of keywords, authors, and comment fields. The outcome of searches will generate files for immediate or future printing.

DELPRO Manual. A manual was written which describes the hardware and software requirements, the installation of hardware and software, the sign-on procedures, and the procedures for establishing the computer accounts necessary to access DELPRO from a Macintosh.





# ANNUAL REPORT

October 1, 1988 through September 30, 1989

## ANIMAL HEALTH AND CARE SECTION, OD, DIR

National Institute of Neurological and Communicative Disorders and Stroke

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## ANNUAL REPORT

### ANIMAL HEALTH AND CARE SECTION, OD, DIR

National Institute of Neurological and Communicative Disorders and Stroke

October 1, 1988 through September 30, 1989

E. CHRISTOPHER STALEY, D.V.M., Chief

The Animal Health and Care (AHC) Section oversees the health needs and provides support in care for animals used in the research efforts of investigations in the NINCDS, Division of Intramural Research. It fulfills these goals by (1) ordering animals from licensed and reliable dealers; (2) housing and providing care for the animals in accordance with the NIH guidelines; and (3) observing the animals and providing health care for those in need.

The Section also serves in an advisory capacity by informing investigators on current guidelines for humane care and use of animals and on such matters as proper anesthetics, best species or strain of animals for particular studies, appropriate surgical procedures, etc. The guidelines used comply to the standards set forth in the Guide for the Care and Use of Laboratory Animals.

The Chief serves as the Section Head and is a member of the NINCDS, Animal Care and Use Committee (ACUC). In this capacity, he utilizes his expertise to advise and guide the Committee in establishing and implementing the Institute's goals for animal care and use practices in conjunction with the functions of the Committee. After approval by the ACUC, research protocols are filed and monitored for compliance by the AHC Section. Ordering policies are established by the AHC Section, and duties include to verify that all protocols have been reviewed, and to provide sufficient space and proper caging prior to the placement of orders for animals. An additional responsibility of the Chief is to organize and coordinate the semiannual review of the NINCDS animal care and use program facilities by the ACUC. Within the AHC Section, animal care technicians are organized as a single service entity under the supervision of the Chief.

Major tasks undertaken by the Animal Health and Care Section this Fiscal Year:

I. Opening and staffing for the new intra agency animal facility in Building 36.

This is a shared facility with the NIMH, NINDS, NIDCD, and the NHLBI, which required the development and implementation of a complete set of standard operating procedures prior to the opening of the facility. All equipment was received and operating procedures were written as planned. The facility opened without delay or complications.

II. Provide consultation and direction for the development of animal facilities for NINDS at the NIH and the contracted research facilities.

Our ultimate goal is to obtain Institute facilities that adhere to the requirements for accreditation by the American Association of Accreditation of Laboratory Animal Care. This is a continuing requirement that is anticipated to continue into FY91.





ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Biophysics  
Basic Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke  
Gerald Ehrenstein, Ph.D, Acting Chief

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## Annual Report

October 1, 1988 through September 30, 1989

Laboratory of Biophysics

National Institute of Neurological Disorders and Stroke

Gerald Ehrenstein, Ph.D., Acting Chief

A major theme of our work is the role of intracellular calcium in triggering important cellular processes, such as exocytosis of secretory vesicles. In particular, we have been studying mechanisms for increasing internal calcium concentration and mechanisms by which the increased calcium concentration triggers exocytosis.

We have developed a working hypothesis for the mechanism of calcium-dependent exocytosis of secretory vesicles that has guided a number of experiments. According to this hypothesis, calcium-activated cation channels are located in the part of the vesicle membrane apposed to the plasma membrane. An increase in intracellular calcium causes these channels to open, and therefore causes a flow of potassium ions into the vesicle. This ionic flow depletes the potassium ion concentration in the intermembrane space, leading to a decrease in the osmolarity of the space. The decreased osmolarity causes a flow of water out of the space and causes the two apposed membranes to move closer together. It is this moving together of the two apposed membranes that triggers membrane fusion and exocytosis.

According to our working hypothesis, secretion should be correlated with the opening of calcium-activated cation channels. Analysis of the literature indicates that this is the case for typical secretory cells with sigmoidal calcium dose-response curves. Since it is known that the calcium dose-response curve for secretion by parathyroid cells is unique, we have examined its calcium dose-response curves for both secretion and the probability that calcium-activated cation channels are open. We now have data for secretion based on experiments with electroporabilized cells. Our data indicate that the dose-response curve is biphasic, with a peak at about  $10^{-7}$  molar calcium, in good agreement with our previous results on the dose-response curve for the probability that calcium-activated cation channels are open in parathyroid cells. This correlation is consistent with our working hypothesis. We are currently testing whether blocking the calcium-activated cation channels of chromaffin cells (where blocking can be achieved with relatively low doses of blocking agents) also blocks secretion. In addition, we are examining vesicle-bound calcium-activated cation channels that have been reconstituted into lipid bilayers.

A very convenient method for measuring properties of neurotransmitter secretion involves the use of a giant synapse. This approach has been limited to the squid giant synapse, and hence to invertebrate synaptic terminals. In order to expand this approach into the domain of vertebrates, we have developed a technique, based on enzymatic digestion, to isolate single neurons with attached presynaptic calyces. These calyces are more than 20 micrometers in diameter. We have used the patch clamp technique to record the calcium current from individual calyces, and are now in the process of determining whether this current is associated with calcium-dependent transmitter release.

Calcium also plays a key role in the process of fertilization. An increase in the cytosolic calcium concentration is known to trigger activation of a fertilized egg, but the molecular trigger for the increase in cytosolic calcium has not been identified previously. We have now shown that the primary messenger from sperm that causes an increase in the cytosolic calcium concentration of eggs is inositol trisphosphate. Thus, the molecule that is known to play the role of a second messenger in many cells, perhaps including egg cells, also has the role of primary messenger in fertilization. We have identified a number of advantages that this confers on the fertilization process.

We have recently established a project to examine electro-mechanical transduction in outer hair cells from the organ of Corti. These cells exhibit elongation and contraction in response to an externally applied electric field. To test whether this response depends on the membrane potential or on the intracellular electric field, we used digitonin to shunt the membrane resistance and apply most of the electric field across the cytoplasm. The application of digitonin abolished the mechanical movement of the cell, indicating that it is the membrane potential that regulates the cellular movement. Preliminary observations with a calcium indicator dye suggest that the cellular movement also depends on the cytosolic calcium concentration. It is likely that in this system calcium acts as a second messenger.

In our project on ionic conductances in nerve and heart cells, the emphasis has been on determining conditions for inducing spontaneous, autonomous firing activity in the squid giant axon. We found that increasing the internal pH to 8.5 or higher causes the axon to fire spontaneously for relatively long periods of time. This behavior should prove interesting for comparison with electrical activity in heart cells.

We have been studying the secretory process in microglia from a point of view that is quite different from the emphasis on mechanism described above. In this project, we are particularly interested in the amounts of particular secretions from microglia and



the possible direct and indirect impacts of these secretions. We previously found that microglia secrete superoxide radical in response to certain chemical stimuli, and that this causes an oxygen burst. We have now investigated this secretion quantitatively and have compared the quantity of superoxide secreted by microglia of normal mice with that secreted by microglia of trisomy 16 mice. We found that the secretion from microglia of trisomy 16 mice is very much larger than from microglia of normal mice subjected to the same chemical stimuli. We are now investigating possible reasons for this dramatic difference. This difference may have important implications for certain neurological diseases. Trisomy 16 in mice is analogous to trisomy 21 in human Downs Syndrome patients. Since neurons and synapses can be functionally damaged by oxyradicals, it is possible that overproduction of these radicals could impair the functioning of the central nervous system.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02087-16 LB

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: W. J. Adelman, Jr., Ph.D.

Chief

LB NINDS

Other: D. L. Butler

Biological Aid

LB NINDS

## COOPERATING UNITS (if any)

University of Minnesota (J. Fohlmeister); Marine Biological Laboratory,  
Woods Hole, MA

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

Section on Neural Membranes

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS.

0.3

## PROFESSIONAL:

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

\*This project has been terminated because of the retirement of the Principal Investigator (April 1989).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02088-16 LB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Function and Structure of Membrane Ionic Channels

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Ehrenstein, Ph.D. Research Physicist LB, NINDS

Others: K. Iwasa, Ph.D. Special Expert LB, NINDS  
N. Moran, Ph.D. Visiting Associate LB, NINDS

## COOPERATING UNITS (if any)

Weed Science Laboratory - AEQI, Dept. of Agriculture, Beltsville, MD.  
(C. Baire and C. Mischke)  
University of Connecticut (R. Satter)

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has already been established that the mechanism for movement of plant motor cells involves the entrance of calcium ions into the cells, but it has not previously been determined by what means the calcium ions enter the cells. We have now identified a cation channel that meets the criteria to be the calcium transporter. This channel is permeable to calcium ions and opens at relatively hyperpolarized potentials.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02092-16 LB

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Subcellular and Extracellular Structure Associated with Nerve and Muscle

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: W. J. Adelman, Jr.

Chief

LB, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

Section on Neural Membranes

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

\*This project has been terminated because of the retirement of the Principal Investigator (April 1989).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02218-14 LB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Drugs on Voltage-Dependent Ionic Conductance in Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|        |                      |                       |           |
|--------|----------------------|-----------------------|-----------|
| P.I.:  | D. L. Gilbert, Ph.D. | Research Physiologist | LB, NINDS |
| Other: | E. F. Stanley        | Senior Staff Fellow   | LB, NINDS |

## COOPERATING UNITS (if any)

Georgetown University, Washington, D.C. (C. Colton, J. Yao, T. Behar);  
CNRS, Marseille, France (L. Fagni); Johns Hopkins University, Baltimore, MD  
(M. Oster-Granite).

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.3

## PROFESSIONAL:

1.1

## OTHER:

0.2

## CHECK APPROPRIATE BOXES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Experiments have been performed on microglia, the resident macrophage in the central nervous system on the trisomy 16 mouse, which serves as an animal model of humans afflicted with Downs Syndrome (trisomy 21). These microglia exhibited an enhanced secretion of superoxide radical anion compared to controls.

In other experiments, we have demonstrated that the squid giant synapse is sensitive to the toxic effects of the reactive oxygen species. The oxygen species tested were hydrogen peroxide and the superoxide radical anion.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02526-08 LB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gated Ionic Channels in Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. F. Stanley, Ph.D. Staff Physiologist LB, NINDS

Other: A. Atrakchi, Ph.D. Visiting Fellow LB, NINDS

## COOPERATING UNITS (if any)

LNN, NICHD

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.9

## PROFESSIONAL:

0.8

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our analysis of calcium current inactivation in rat intermediate lobe cells showed that the amplitude of the calcium current in these cells is strongly dependent on the prior history of the membrane potential. The calcium channel inactivates at a very slow rate that has not been described previously. This finding is of biological importance since it indicates that secretion may be modulated in these cells simply by regulating the cell resting membrane potential.

A more detailed understanding of these slow changes in calcium channel activity will be examined by analysing channel inactivation at the single channel level. Single calcium channels will be characterized on the intermediate lobe cells in tissue culture. The changes of channel kinetics with prolonged depolarizations will be recorded and the changes in channel open probability will be compared to the previously published time course of calcium current inactivation.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02606-06 LB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Chemical Transmission at the Squid Giant Synapse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. F. Stanley, Ph.D. Senior Staff Fellow

LB, NINDS

Other: G. Goping, Technician

LCBG, NIDDK

## COOPERATING UNITS (if any)

LNN, NICHD

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.4

## PROFESSIONAL

0.3

## OTHER

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The large presynaptic nerve terminal at the squid giant synapse is unique as an experimental preparation in that it is possible to determine the physiological events involved in the release of neurotransmitters. It has not as yet been possible to carry out similar studies on vertebrate synapses due to the lack of a suitable experimental model. We have explored the possibility of using the giant calyx-type synapse in the chick ciliary ganglion as such an experimental model.

We have developed a technique, based on enzymatic digestion, to separate individual ciliary neuron 'capsules' (the neuron, the presynaptic calyx nerve terminal, and the ensheathing Schwann cells) from the ciliary ganglion and to strip the Schwann cells from the outer capsule layer. This leaves the calyx presynaptic nerve terminal accessible to direct experimentation. We have used the patch-clamp technique in the whole-cell mode to record calcium currents directly in the calyx. This is the first reported recording of a calcium current directly from a vertebrate presynaptic nerve terminal.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02608-06 LB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Comparative Aspects of Ionic Conductances in Nerve and Heart Cell Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. R. Clay, Ph.D.

Physicist

LB, NINDS

## COOPERATING UNITS (if any)

McGill University (A. Shrier).

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.1

## PROFESSIONAL:

1.0

## OTHER:

.1

## CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with a comparative analysis of ionic current channels in nerve and heart cell membranes and the relationship of these channels to electrical excitability in both preparations. During the past year the primary experimental preparation which has been used is the squid giant axon. This work has focussed on the mechanisms required to induce spontaneous, autonomous firing activity in the squid axon. A major finding has been that an increase in the internal pH to pH 8.5, or higher is sufficient to cause the nerve to fire spontaneously. This effect is mediated by a shift of the potassium channel inactivation curve along the voltage axis by the increase in pH, which hyperpolarizes the membrane, thereby reducing sodium channel inactivation.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02609-06 LB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Egg Activation Following Fertilization

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                    |            |
|---------|----------------------|--------------------|------------|
| PI:     | K. Iwasa, Ph.D.      | Special Expert     | LB, NINDS  |
| Others: | G. Ehrenstein, Ph.D. | Research Physicist | LB, NINDS  |
|         | J. Russell, Ph.D.    | Research Chemist   | LNN, NICHD |

## COOPERATING UNITS (if any)

Emory University, Atlanta, GA (L. DeFelice)

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously demonstrated that a fertilization membrane forms around a sea urchin egg when it is injected with a soluble spermatozoa fraction isosmotic with seawater. We have now found that a spermatozoon contains inositol trisphosphate at such a high level as to trigger calcium elevation in the egg leading to the exocytosis of the cortical granules and the elevation of the fertilization membrane. Thus, inositol trisphosphate plays the role not only of a second messenger within eggs, as in other cells, but of the primary messenger from spermatozoa to eggs at fertilization.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02709-04 LB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Secretion of Neurotransmitters and Hormones

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                     |            |
|---------|----------------------|---------------------|------------|
| P.I.:   | G. Ehrenstein, Ph.D. | Research Physicist  | LB, NINDS  |
| Others: | E. F. Stanley, Ph.D. | Senior Staff Fellow | LB, NINDS  |
|         | K. Krebs, Ph.D.      | Staff Fellow        | LB, NINDS  |
|         | S. Pocotte, Ph.D.    | Staff Fellow        | LB, NINDS  |
|         | J. Russell, Ph.D.    | Research Chemist    | LNN, NICHD |

## COOPERATING UNITS (if any)

LNN, NICHD

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.0

## PROFESSIONAL:

2.8

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

We have developed a model for calcium-dependent secretion based on the opening of calcium-activated cation channels present in secretory vesicles. One test of the model is whether secretion is correlated with the opening of these channels. Most secretory cells have sigmoidal calcium dose-response curves for secretion. A few measurements of the calcium dose-response curve for the open probability of calcium-activated potassium channels have been made, and these correlate with secretion dose-response curves.

Parathyroid cells have a unique calcium dose-response curve for secretion. Secretion decreases when calcium concentration is increased. We have examined the channel properties and the secretion properties of bovine parathyroid cells in order to determine whether they are correlated in this unusual cell. We have now found that the dose-response curve for secretion is biphasic, with a peak at about  $10^{-7}$  molar calcium. We have previously shown that the open probability for the calcium-activated potassium channel in parathyroid cells is also biphasic, with a peak at about  $10^{-7}$  molar calcium. Taken together, these results support our hypothesis that the opening of these channels is required for secretion.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02799-01 LB

## PERIOD COVERED

October 1, 1988 through September 3, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electro-Mechanical Transduction Mechanism in Outer Hair Cells

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. Iwasa, Ph.D.

Special Expert

LB, NINDS

Other: B. Kachar, M.D.

Visiting Scientist

LMO, NIDCD

## COOPERATING UNITS (if any)

John Hopkins University, Baltimore, MD (W. E. Brownell)  
LMO, NIDCD

## LAB/BRANCH

Laboratory of Biophysics, BNP, DIR

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.7

## PROFESSIONAL:

0.6

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The fast mechanical response is believed to be the cellular basis of the positive feedback mechanism required for the fine tuning process of the hearing organ. We observed that the reduction of the ionic strength of the internal medium in the whole cell recording configuration eliminated the fast response. This confirms that the fast response is not attributable to the electrokinetic effect and eliminates the possibility that it is an artifact at the patch pipette. We demonstrated that the fast mechanical response of the outer hair cell is membrane potential dependent, using externally applied electric field with and without the presence of membrane permeabilizing agents. To clarify further how a change in the membrane potential causes mechanical displacement of the cell, we are currently examining a hypothesis that calcium ion is involved in the fast response as the second messenger. So far we obtained some pieces of evidence which support the hypothesis.









# ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Central Nervous System Studies

National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT  
Laboratory of Central Nervous System Studies  
October 1, 1988 – September 30, 1989

The elucidation of the pathogenesis of dementing brain amyloidoses, both the transmissible type (such as kuru, scrapie, and Creutzfeldt-Jakob diseases, caused by slow, unconventional viruses) and the nontransmissible type (such as normal aging, Alzheimer's disease, and Down's syndrome) continues to be our primary area of inquiry.

Our concept that the formation of brain amyloid from a normal host precursor protein underlies the pathogenesis of Alzheimer's disease (AD) has provoked considerable molecular study of brain amyloid during the past three years. We were the first to characterize the gene for this aging brain amyloid precursor protein (b-protein; A4 protein), to locate it on chromosome 21 of man and 16 of mouse, and to show its high conservation in evolution. This gene has turned out to be identical to that for an excreted rapidly turned-over protein which specifically binds to the gamma-subunit of nerve growth factor and many other serine proteases and cell modulators which contain such serine protease as binding regions. This important negative feedback control loop may explain the rapid synthesis, short half-life and wide distribution in neurons of this brain amyloid precursor. Our work showing that the gene was expressed in several alternatively spliced forms, with and without a 57 bp or a 76 bp insert which specify a serine protease inhibitor, now fits well with the identity of protease nexin II and the alternatively spliced forms of our amyloid precursor protein containing the serine protease inserts.

Our studies of mRNA expression in different brain cells of normal juveniles, aging brain and AD brains have revealed that all neurons which develop neurofibrillary tangles (NFT) and are most vulnerable to loss in aging and in AD carry a very high level of turned-on message. Not all cells with high levels of amyloid b-protein mRNA develop NFT, and thus its high expression appears to be a necessary, but not a sufficient, condition for NFT formation. Interestingly, in Guamanian ALS/PD typical NFT appear in large motor neurons which contain a high, up-regulated message. We have also studied the regulation of mRNA and the precursor protein expression in hippocampal neurons and in endothelial cells *in vitro*. Thus, continued molecular and cell biological studies of brain amyloid b-protein biosynthesis, processing and regulation will surely continue to dominate research on AD and aging brain for some time.

The discovery that the subunit of aging brain amyloid in AD and Down's syndrome had no amino acid homology with the much larger subunit of scrapie-kuru-CJD amyloid in SAFs or kuru-plaques led to the clear differentiation of transmissible from non-transmissible cerebral amyloidoses. Scrapie and the transmissible dementias (kuru, CJD and its GSS variant) form the amyloid of SAF and scrapie or kuru plaques from a proteolytically cleaved portion of the larger infectious form of the scrapie amyloid precursor protein. The genes for the brain amyloid precursor and for the scrapie amyloid precursor show no sequence homology and the amyloidogenic subunits cleaved from the full length precursors are extremely different. The subunit protein for the non-transmissible brain amyloidoses of aging and AD is a polypeptide of 4.1 kDa (42 amino acids) in size and is nonglycosylated; that from scrapie-kuru-CJD is 27 kDa in size and has two glycosylated sites. The genes for the precursor is on chromosome 21 in man for the former and for the latter on chromosome 20 (in mice they are on chromosome 16 and 2, respectively).

Our repeated assertions that the unconventional viruses contain no non-host proteins and were replicating polypeptides now appear to be vindicated by much more work from our own and many other laboratories. It also appears that the scrapie amyloid precursor protein is converted to an infectious form by configurational changes in the secondary and tertiary

structure of the normal scrapie precursor protein (SPP). On inoculation of susceptible hosts the scrapie monomer autonucleates and autopatterns this conversion of the normal, non-infectious host SPP to the infectious form. We also believe that most sporadic case of CJD arise by de novo spontaneous conversion of the normal SPP to the infectious form, a rare event occurring at the frequency of one per million persons per year (the annual incidence of CJD throughout the world). In the familial forms of CJD and GSS where the occurrence is an autosomal dominant trait, we have found that each family has one of four different mutations, each causing a single amino acid change. This causes a million-fold increased probability of the spontaneous configurational change to an infectious polypeptide, and thus appears as an autosomal dominant trait.

We have established a multifaceted research program to investigate the pathogenetic mechanisms underlying the brain amyloidoses. These studies include in vitro study of amyloid fibrils formation from synthetic polypeptides. Using the unique resource of our tissue bank of specimens from experimental animals we have initiated studies on the structure of the amyloid protein in different strains of virus from the same host. Virus properties of pathogenesis, incubation time and host range must depend on secondary and tertiary configurational changes in a host precursor if no pleomorphism in amino acid sequence can be shown between virus strains from a single breed of host.

Recent experimental data from our laboratory confirms our hypothesis that the amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PD) in high-incidence foci in the Western Pacific is caused by a pathogenetic elemental deposition in the central nervous system which results from severe calcium and magnesium deficiency. ALS-like neurofibrillary tangles have been produced in cynomolgus monkeys and rabbits and in vitro in motor neurons by chronic aluminum toxicity. This confirmation of the mineral deposit etiology in Guamanian ALS/PD has stimulated a renewed worldwide interest in these high incidence foci in the Western Pacific. Interestingly we have never been able to give serious credence to extensive claims that cycad toxicity was involved in the high incidence ALS/PD foci. This skepticism is now vindicated.

Research work on acquired immunodeficiency syndrome (AIDS) and related topics continues to occupy a significant portion of our resources. This includes studies on pediatric and adult encephalitis caused by human immunodeficiency virus (HIV), a major cause of encephalitic death in the United States today. We also are conducting studies in nonhuman primates to develop an experimental animal model for AIDS, and to evaluate antibody response produced by potential vaccines against HIV infection.

Our research on other human retroviruses, particularly human T-cell leukemia/lymphoma virus type I (HTLV-I), increases in depth and scope. This includes intensive investigation of an encephalomyelopathic syndrome called, variously, tropical spastic paraparesis, Jamaican neuropathy, Pacific spastic paraparesis, and HTLV-I-associated myelopathy. Our virological, immunological, clinical, and epidemiological research points to HTLV-I as the primary cause of this syndrome, which occurs in high-incidence foci of HTLV-I infection worldwide.

Our work on the hantaviruses, causative agents of hemorrhagic fever with renal syndrome, continues as a worldwide collaborative effort, involving groups in Europe and Asia. This year, however, we have concentrated on finding domestic manifestations of hantavirus infection, and on developing experimental models of Prospect Hill and Puumala virus infection in primates.

We continue our studies on the mechanism of language acquisition, including the naturalistic observation of extreme polylinguality. Our comparative inquiries on widely

divergent styles of psychosexual development in children from diverse cultural milieus continue to yield new data on neurological programming which departs from "normal" behavior much further than previously imagined by most psychiatrists, psychologists, sociologists, and anthropologists.

We have always studied not only the clinical and laboratory aspects of neurological syndromes, but also the social and public health implications of these syndromes. Our long-term data gathering and analysis activities concerning kuru in Papua New Guinea, ALS/PD in Guam, and Viliuisk encephalomyelitis in the Soviet Union, as well as others, continue to provide valuable insights on cultural reaction to ongoing epidemic and endemic diseases, and to suggest available social alternatives. Much that we learn is applicable, by extrapolation, to contemporary problems we face in the United States, such as Alzheimer's diseases and senile dementias, the AIDS epidemic, and the seemingly uncontrollable illicit use of drugs.

#### Slow Unconventional Viruses Causing Transmissible Brain Amyloidoses

Our laboratory has concentrated its main effort on elucidating the relationship between the viruses of kuru, Creutzfeldt-Jakob disease (CJD), and scrapie and their host-specified precursor proteins. We now know that scrapie virus is the monomeric form of the configurationally changed 35-37 kDa scrapie precursor protein present in normal brain and in infected brain tissues, but modified by infection to a less soluble protease-resistant form which is infectious. The 27-30 kDa scrapie amyloid protein (prion protein; PrP<sup>27-30</sup>), which assembles *in vitro* into congophilic, birefringent rods resembling the scrapie-associated fibrils (SAF) of Merz and into kuru-CJD-scrapie plaques is also infectious, even as a monomer.

We have now demonstrated that this normal host protein modified by scrapie virus infection is itself the infectious agent (an amyloid molecule, autoinducing the modification of host protein precursor into its infectious form). No non-host nucleic acid has been demonstrated, even in highly infectious preparations. The improbable conjecture that the entire infectious process is that of autonucleated and autopatterned conformational change of a protein precursor which leads to a crystallization and polymerization forming amyloid fibers of SAF and kuru plaques is now verified. The host gene specifying the 35-37 kDa precursor protein has been fully sequenced. It is on chromosome 20 in man, and 2 in mice.

Polyclonal and monoclonal antibodies prepared against synthetic polypeptides of the N-terminus of the amyloid of scrapie reveal varying distribution and patterns of the epitopes in normal and infected tissues. Such antibodies have shown reactivity to the scrapie-associated proteins and, to our surprise, to many purified proteins, including purified natural and synthetic human growth hormone. However, these antibodies to SAFs or to the synthetic polypeptide specifically label purified SAFs from kuru-, CJD- and scrapie-infected brains. Such SAFs are not obtainable from brains of other human neurodegenerative diseases, and thus this new immunological gold-labeling technique has been used to identify serologically the SAFs in the two patients with frozen brain available of the four patients who developed CJD from injections of contaminated human growth hormone preparations.

These immunocytochemical and molecular biological studies on the scrapie/kuru/CJD-associated proteins and their normal precursors are largely aimed at preparing them in high purity and sufficient amounts for crystallographic study, and investigation at the organic chemical level, of the fine structural modification involved in the conversion of normal host-protein into amyloid fibers which appears to be the major pathogenic reaction of these diseases.

For over two decades we have carefully saved at <-70°C frozen tissue from chimpanzees and other nonhuman primates affected with the human Creutzfeldt-Jakob disease and Gerstmann-Sträussler syndrome viruses, kuru virus, and scrapie, and the frozen tissues collected from cats, hamsters, guinea pigs and other animals susceptible to these viruses. These were collected and saved for eventual biochemical study when this would be possible. It is now possible to process these tissues for PrP<sup>27-30</sup> protein, for



SAFs, and for the 33-35 kDa scrapie-specific protein and its precursor. As a more sophisticated study of the structure of these proteins is possible, we hope to determine from this material the contribution of the host to these subacute spongiform encephalopathy viruses or slow unconventional viruses. This we are in a unique position to do, since it would take from two years to over a decade for other laboratories to obtain infected brain material from a number of different species each inoculated with the same strain of virus.

We have indications that there are many strains of CJD viruses. Using these tissues, it is possible to answer the critical question of the relative contributions of the host and the virus strain to the pathogenesis of the disorders and the molecular structure of the virus strains. Since we expect all strains or passage lines of kuru-CJD-scrapie viruses to replicate by an infectious transformation of the normal host precursor protein to an infectious configuration, it follows that all virus strains should produce progeny in a given host which have the identical host amino acid sequence in the infectious monomer. Strain differences determined by the host precursor gene or its mutations would not "breed true", i.e. they would be carried into the progeny. Thus, if we do find strain differences in viruses from the same host, this would require a different explanation than conservation of genotypic identity. Rather, we should expect a conservation of secondary and tertiary configurational change by autonucleation and autopatting epitaxial crystal replication and growth. The stored frozen brain passage material, particularly of different viruses (scrapie-kuru-CJD-GSS) passed into the same breed of host, is being used for resolution of this critical matter.

#### Non-transmissible Brain Amyloidoses of Aging, Alzheimer's Disease and Other Dementia

Amino acid sequencing of the 4 kDa polypeptide subunit of the paired helical filaments of neurofibrillary tangles (NFTs), of amyloid plaque cores, and of amorphous amyloid in congophilic angiopathy indicates that all three pathognomonic structures of the aging brain, Alzheimer's disease, Pick's disease, progressive supranuclear palsy, late Down's syndrome, Guamanian ALS/PD and Von Economo's encephalitis are composed of identical 4 kDa (42 amino acids) subunits. In the preparations of purified PHF from NFTs of Guamanian ALS and PD, no extracellular amyloid in the form of amyloid plaques or vascular amyloid deposits were present to produce possible contamination. This 4 kDa polypeptide subunit which easily associates into dimers, tetramers, octamers, and hexadecamers, shows no amino acid sequence homology to the infectious scrapie amyloid subunit of the transmissible cerebral amyloidoses.

#### Hippocampal Neuronal and Endothelial Cell Cultures for In Vitro Study of b-Amyloid Precursor Gene Regulators

Both vascular endothelial cell and hippocampal neurons in cell culture have been used for studies of in vitro expression of the amyloid b-protein precursor and also for the modulation of mRNA expression of this precursor by growth factors, interleukin-1 and other regulatory molecules. This work obviously has important implications for preventative and therapeutic intervention in the over-expression of the precursor.

#### The Carbohydrate of Glycosylated Amyloid Subunits

Enzymatic removal of q and N-linked carbohydrates from the infectious scrapie amyloid or the infectious form of SPP does not diminish the infectivity titer. Thus, glycosylation is not critical for virus replication.

#### In Vitro Production of Amyloid Fibrils

We have been studying the re-coiling of polypeptide chains into different fine structural configurations which permit beta-pleated sheet stacking into fibrils resembling the amyloid fibrils of human pathology. Thus, using b-2-microglobulin we have succeeded in producing fibers that have the congophilic and green birefringent properties of amyloid fibrils and also the electron microscopic appearance of such fibers and even paired twisted fiber structures. More specifically, we have produced amyloid-like fibers from potential precursors of brain amyloids, such as the 200 kDa protein of neurofilament, and are trying with MAP-tau.

The 200 kDa neurofilament protein assembles in vitro into amyloid-like filaments which are both congophilic and green birefringent and in morphology resemble amyloid fibrils; the amyloid-like properties increase on partial cross-linking with paraformaldehyde fixation.

The possibility that the fine structural changes of the aging or diseased brain may be reproduced in vitro is obviously intriguing.

#### Neurofilament Pathology in Human Neurodegenerative Disease

The gene encoding the amyloid b-protein has been shown to be highly conserved in evolution and is expressed in various human and animal tissues. As a complex transcriptional unit, it utilizes alternative splicing; alternative spliced forms of the amyloid b-protein precursor cDNAs contain 50% homology to the Kunitz family of serine protease inhibitors. It may be the absence, inhibition or overexpression of these alternative forms that modify the host precursor proteins leading to the production of amyloid b-protein.

These modified forms of the amyloid b-protein in its microfibril or oligomeric forms, like the fibril amyloid enhancing factor (FAEF) in AA amyloidoses, could act as amyloid enhancing factors, or as nucleants or niduses that accelerate its own formation by self polymerization and copolymerization with other molecules like glycosaminoglycans which leads to amyloid deposition.

In Alzheimer's disease and Guamanian parkinsonism-dementia, the 42-amino acid subunits of the amyloid of the neurofibrillary tangles, amyloid plaque core, and congophilic angiopathy, could themselves serve, in the form of oligomers or fibril microfragments, as nuclei that enhance their deposition as amyloid.

#### Creutzfeldt-Jakob Disease and Human Growth Hormone

A further area of intense involvement of our laboratory has been in the problem of Creutzfeldt-Jakob disease in recipients of human growth hormone (HGH) prepared from pooled autopsy pituitary glands by the NIH and other programs. Ten patients are now known who developed CJD from infected or contaminated hormone. At least three different batches of pituitary glands have been contaminated, since two patients occurred in England and one in New Zealand (where none of the American products were used) and at least one batch was used by all seven American patients. Incubation periods have ranged from 4 to 20 years, and in frozen brains from two cases we have been able to demonstrate the PrP 27-30 protein by Western immunoblotting, and SAF by immune electron microscopy, using gold-labeled antibody. Moreover, in another HGH recipient with motor neuron signs, who was suspected of having CJD, failure to detect PrP 27-30 protein provided strong evidence against the diagnosis of CJD, and verified the subsequent neuropathological diagnosis of ALS. Primates have been inoculated with more than 50 separate batches of HGH, but incubation periods may be up to five years. Intense surveillance is under way of some 8000 other young people who received hormone injections, and one further probable case has been identified. An Australian case has followed use of CJD-contaminated pituitary gonadotropin.

#### Toward a Biochemistry of Silicon and Aluminum

The metabolic adjustment to severe environmental deficiency of calcium and magnesium which is responsible for the deposition of calcium, aluminum, silicon, phosphorus and other minerals in brain cells in early life in the high-incidence foci of amyotrophic lateral sclerosis (ALS) and parkinsonism dementia (PD) and the early appearance of Alzheimer's neurofibrillary tangles (NFTs) in isolated populations in the Western Pacific (Guam, Japan, West New Guinea) was first suggested by epidemiological and ecological studies. Mineral analyses of environmental specimens of soil and water confirmed this hypotheses. Finally, electron probe X-ray microanalyses, using both energy-dispersive and wavelength-dispersive spectrometry, has demonstrated these long-term deposits in NFT-bearing hippocampal neurons of Guamanian ALS and PD patients and of normal individual exposed to the same



environmental deficiencies. When these  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  deficiencies are removed by increased access to outside foodstuffs, changed water supply, and improved transportation and economy, all three diseases (Alzheimer's NFTs, ALS and PD) have declined markedly in incidence or disappeared within a period of two or three decades.

This discovery of the primary cause of all three pathological processes in the Western Pacific isolates has led to animal experiments which further substantiate the hypotheses (see below) and stimulated a renewed interest in the role of mineral deposition in interfering with axonal transport. Even therapeutic and prophylactic clinical regimens are now suggested and some are under study.

Furthermore, the role of silicon and its polymers in altering the secondary structure of proteins through long series of hydrogen bonds is now under investigation. Silicon and aluminum compounds can interact strongly with phospholipids, lipids, carbohydrates and oligonucleotides as well as with polypeptides. Thus, mineral deposits of montmorillonite clays-calcium-aluminum-silicates-and hydroxyapatites can denature and alter protein fine structure and conceivably play an active role in degradation of host precursor proteins to amyloids.

The recent confirmation of older observations of silicon-containing deposits in the center of purified insoluble amyloid plaque cores from Alzheimer's disease patients and in Alzheimer's NFTs has greatly stimulated interest in the possible role of these silicon and aluminum-containing mineral deposits as nucleating agents or even as autocatalytic agents in the deposition or crystallization of such amyloid deposits. The work and thinking of this laboratory in these directions has had a major impact on determining the course of modern inquiry into aging and the degenerative amyloidoses of brain, including Alzheimer's disease.

#### Role of Low Dietary Ca and Mg in the Evolution of Motor Neuron Disease

Oral administration of a low calcium and magnesium diet to young cynomolgus monkeys (*Macaca fascicularis*) for nearly 4 years has induced degenerative changes and variable degrees of intracellular calcium accumulation in the motor neurons of the spinal cord and brainstem, and in the giant Betz cells of the cerebral cortex. Supplementation with low dose aluminum and manganese chloride has resulted in a cellular accumulation of argentophilic material of neurofilament origin in different areas of the central nervous system. None of the animals, however, showed overt clinical signs despite these neuropathological changes.

Immunocytochemical staining, using monoclonal antibodies against neurofilaments, has revealed an aberrant accumulation of the phosphorylated form of the 200 kDa subunit protein within the perikarya of motor neurons in the spinal cord, mesencephalic component of trigeminal nucleus, zona compacta of substantia nigra, and of large pyramidal neurons in the cerebral cortex. This abnormal accumulation was noted maximally in animals fed the low-calcium and magnesium diet supplemented with aluminum.

In addition to the neuronal pathology, axonal spheroids were seen both in the neuropil of the nuclear area and white matter. As in ALS, the lateral and anterior columns of the spinal cord, the spinocerebellar tracts and corticospinal tracts in the brain stem revealed axonal swellings, spheroid formation and focal axonal loss. Gliosis was conspicuously absent.

These observations support the hypothesis that low  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  levels interfere with axonal transport of the neurofilament subunits. This is further accentuated by the addition of aluminum. It is believed that the compact, relatively rigid molecules of phosphorylated 200 kDa neurofilament proteins accumulate in the neuronal soma leading to functional derangement and eventually to cytolysis.

This nonhuman primate model provides a means to understand the pathogenetic mechanism involved in the evolution of lesions in motor neuron disease.

Fetal Motor Neuron and Hippocampal Neuron Cell Cultures for In Vitro Studies of Neurofilament Synthesis, Catabolism and Toxic Reaction to Aluminum

Within the past year a program of in vitro neurobiological studies employing monolayer cultures of dissociated fetal neuronal cells has been reintroduced to the laboratory. The impetus underlying these studies is the observation of the differential morphological responses amongst varying neuronal populations to heavy metal neurotoxins. Exemplifying this is the virtual absence of neurofilamentous pathology in rabbit hippocampal neurons exposed to intracisternally administered aluminum salts, contrasted to the accompanying extensive destruction of anterior horn cells--the hallmark of which is aberrant neurofilamentous accumulations. The in vitro extension of these observations is providing a new understanding of the mechanisms regulating neurofilament expression. This has necessitated the development of a novel technique employing isopycnic centrifugation in Percoll step gradients for the purification of motor neurons from fetal mouse and rabbit spinal cord homogenates. These neurons co-cultured with muscle fibers, have been successfully maintained for prolonged periods in serum-free medium. Parallel monolayer cultures, of dissociated fetal rabbit hippocampal neurons, co-cultured in serum-free medium over astrocytes, have recently been successfully introduced.

Utilizing these cultures, ongoing studies are mapping the coexpression of neurofilaments and neuronal enzymes during the course of normal neuronal maturation in vitro. Employing immunohistochemical techniques, these neuronal components are identified in situ and with sensitive neurobiological assays, their synthesis quantified. Subsequent studies will explore the expression of these elements under aberrant environmental conditions.

Retrovirus Encephalomyelopathic Human Lentivirus (AIDS) in Children and Adults

Our laboratory is also working on the problem of the primary encephalitis which characterizes almost all cases of childhood AIDS acquired congenitally from a human immunodeficiency virus (HIV)- infected mother. HIV-infected mothers are giving birth to infected babies in 80% of their offspring, and some 80% of these infected offspring develop clinical AIDS. Most develop disease within one to two years after birth; a few are as delayed as four to five years of age. All, however, develop a primary encephalitis characterized by dysarthria, speech impairment, with eventual aphasia, and severe midline truncal ataxia and loss of developmental milestones.

We have demonstrated the virus in the brains of these infants by in situ hybridization, by fluorescent antibody localization, and by electron microscopy. In histological studies we have demonstrated that there is a specific neuropathology with large, multinucleated macrophages in the brain, loaded with virus particles that are visible by electron microscopy. Similarly, there are brain cells which appear to be astrocytes, bulging with the human lentivirus particles. In neurons, these virus particles are rarely seen, and when seen are few in number. It was from the awareness of the primary human lentivirus encephalopathy of infants and children that a search was made in the brains of adults, and similar pathology was found in more than half of the fatal cases of AIDS.

It is only in the last five years, therefore, that clinicians have become increasingly aware that many adult AIDS patients show varying degrees of dementia which is not due to opportunistic infection with mycoplasma, mycobacterium, yeast, toxoplasma, cytomegalovirus, or herpes simplex virus.

The human lentivirus has thus become the major cause of encephalitic death among children and adults in the United States. In adults and more frequently in children, primary encephalitis from human lentivirus infection may occur without an immune deficiency syndrome. Thus, we are dealing with another example of transmissible virus infection resulting in a chronic dementia.

#### Search for an Animal Model of AIDS

Our laboratory first demonstrated active infection of chimpanzees with human immunodeficiency virus (HIV) (formerly LAV and HTLV-III) and with primary human blood and tissues obtained from AIDS patients. The animals become seropositive but do not develop clinical disease, and if there is any alteration in immune function, it is a transient lymphocytosis with moderate impairment of lymphocyte function, but not a helper-suppressor ratio change equivalent to that in human AIDS. The animals show no clinical disease five years after inoculation but they remain seropositive and viremic.

Such chimpanzees developing primary infection on inoculation with human brain tissue from AIDS patients provided the first demonstration of the live virus in the brain of AIDS patients. Since such infection has occurred even at high dilutions of suspensions of brain tissue from AIDS, the presumption is that the virus is in brain and in considerable quantity.

Many other species of nonhuman primates have been inoculated without producing disease, or primary infection, or antibody conversion; however, an occasional rhesus monkey inoculated with these human viruses has developed an antibody response. The human lentiviruses do not produce disease in nonhuman primates, even though they are very closely related to simian immunodeficiency virus. Thus, we are without a good experimental model for vaccine evaluation in small animals or in nonhuman primates, and all that can be done at present is to test for the ability of vaccines to protect against primary infection.

Our laboratory first introduced the studies of the Icelandic visna and maedi sheep diseases in the United States at the NINDB Symposium on Slow Viruses in 1962, to which the Icelandic workers were invited as participants. We made the first isolations in the United States of the visna virus, later defined as the prototype lentivirus, from the brain of a sheep with Montana sheep disease. The maedi virus previously was thought to cause only pulmonary involvement in Montana sheep disease. It is now known to be the same virus as visna, which may cause maedi, or zoegersiekte, in Iceland and the Netherlands, respectively, the pulmonary forms of visna virus infection.

AIDS virus (or HIV) belongs to the subfamily Lentivirinae in the Retroviridae family, which includes visna virus, equine infectious anemia virus, and caprine arthritis encephalitis virus. We have found that horses inoculated with the human AIDS lentivirus develop a transient antibody response, but no disease.

#### HTLV-1 Neuromyelopathies

##### (Tropical spastic paraparesis, Jamaican neuropathy, and HAM)

We have continued our studies of Jamaican neuropathy in Jamaica and of Pacific (tropical) spastic paraparesis in the Tumaco area of southwestern Colombia on the Pacific coast. The Tumaco focus has a very uniform disease, and the earlier studies were done by Gajdusek. In these studies we found that patients had a much higher percentage of treponema-positive spinal fluid than control patients, but in our most recent study there has been a striking decline in the rate of positivity, probably indicating that the earlier, not the newer, patients had yaws. On the other hand, we have now demonstrated an IgG antibody response in spinal fluid and serum to human T-cell lymphotropic virus type I (HTLV-I). By ELISA, antibodies against HTLV-I can be detected in cerebrospinal fluids (CSF) from most patients with spastic paraparesis and in none of the controls with other neurological diseases. We confirmed the



ELISA results by Western blot and radioimmunoassay. In the coastal area of Colombia, the seroprevalence rate of HTLV-I infection among normal adults is very low, less than one-tenth that found in TSP patients.

Seroepidemiological studies have now been done, in several other towns on the Pacific coast of Colombia, and new patients with TSP have been found. Family and household studies in Tumaco have shown that relatives of TSP patients living in the same household have a much higher prevalence of antibody to HTLV-I. Whereas sexual and mother-to-child transmission of the virus are recognized, the possibility of transmission by insect vectors has to be explored because of the high percentage of positives found in TSP households. In Jamaica, the disease occurs island-wide, and new patients are being documented each week. Household studies have been done and the results are the same as found in Tumaco. A detailed epidemiological study is now in progress at the University Hospital, Jamaica and this includes the study of patients with other neurological diseases and controls. HLA typing is also being done on TSP patients and these results will be compared with the findings seen in adult T-cell leukemia patients in Jamaica. A protocol for treatment with steroids has been established and these trials have been completed in Jamaica and are in progress in Tumaco. Several isolates of HTLV-I-like virus have been cultured from the blood and CSF of Jamaican and Colombian patients with TSP and characterization of these HTLV-I isolates from blood and CSF samples are underway. Initial results suggest that there are differences in the isolate from TSP and ATL Jamaican patients. HTLV-I-like viral particles have been identified in the fixed spinal cord of a Jamaican TSP patient. In the study of spastic paraparesis in the Seychelles, we have found similar seropositive rates of IgG antibodies to HTLV-I in serum. An identical chronic myelopathy associated with HTLV-I infection has been reported from southern Japan which is a temperate region. At the time of writing last year the initial report from Martinique and our discoveries in Jamaica and Colombian were supported by those from Japan, the Seychelles and Trinidad. We now have confirmation from twenty-six other countries and the occurrence of TSP in Caucasians who have not visited endemic regions has also been reported from the temperate zones of France and Italy. These factors serve to emphasize the need for intensive study of neurological involvement with HTLV-I infections, particularly in view of the fact that antibodies to a virus of this group have been found in cases of multiple sclerosis (MS). The similarity to MS of some of the histopathological changes in Jamaican TSP patients has also provoked the thought that this group of viruses could play a role in producing varied manifestations of one disease process in different ethnic groups and regions: MS in temperate climates and tropical paraparesis in the tropical and subtropical areas. In addition we discovered that almost 90% of Jamaican patients with polymyositis have IgG antibodies to HTLV-I, and these findings were confirmed by Western blot.

We are now studying CSF and sera from MS patients for HTLV-I antibody and frozen MS brain tissues for the presence of genomic sequences, using in situ hybridization and using polymerase chain reaction. We have available fixed spinal cord tissue from several Jamaican TSP patients and muscle biopsy tissue from polymyositis patients. Studies of these and other available tissue by the polymerase chain reaction (PCR) technique have been initiated.

#### High Prevalence of HTLV-I Infection in the Asia-Pacific Basin

In our search for high-prevalence foci of HTLV-I infection in the Western Pacific, we have tested by ELISA and Western immunoblot more than 4000 sera collected between 1956 and 1988 from 37 population groups. High prevalences of antibodies against HTLV-I, ranging from 14% among the Touri to 51% among the Hagahai, were found among the coastal and lowland populations of New Guinea, the Solomon Islands and Vanuatu. Populations in the more isolated interior of Papua New Guinea tended to have no seropositivity, except for the Genatei, a remote highland group which had an 18% seroprevalence before contact with the outside world. Similarly, the more isolated Polynesian outliers of Anuta and Tikopia had low rates and the less isolated outliers of Rennell and Bellona had considerably higher prevalences. As

confirmed by Western immunoblot, HTLV-I seroprevalences in several Melanesian populations were as high as those found in HTLV-I-endemic regions such as southwestern Japan and the Caribbean basin.

The high seroprevalences in Melanesia have been contested because of the inability of many ELISA-positive sera to be confirmed by Western analysis and the failure of such sera to neutralize a prototype strain of HTLV-I. However, with our recent identification and serological verification of a case of tropical spastic paraparesis caused by HTLV-I in a life-long indigene of East Guadalcanal in the Solomon Islands, and our isolation of HTLV-I-like retroviruses from indigenous New Guinean carriers from remote villages, the endemicity of HTLV-I infection in Melanesia is now irrefutable. We have maintained that the high frequency of indeterminate Western immunoblots in Melanesia is indicative of a closely related but distinct retrovirus. Further characterization of our isolates should settle this issue.

Another significant finding has been the identification of HTLV-I encephalomyeloneuropathy among Mestizo and Caucasian in Chile, a temperate zone. HTLV-I, serologically indistinguishable from prototype strains of HTLV-I from the Caribbean basin and Japan, has been isolated from several of these patients. These data augments our concepts of the geographical and ethnic distribution HTLV-I-caused spastic paraparesis.

#### Viliuisk Encephalomyelitis in Yakut People in Siberia, USSR

We have just published a definitive bibliography of references on this disease since most reprints are unfamiliar to English-speaking neurologists. A complete review of this disease in English has been submitted to Brain. A detailed and comprehensive report of Viliuisk encephalomyelitis (VE) is being prepared reporting the unique CNS pathology.

Analysis of clinical descriptions of 248 VE cases and a comprehensive clinical characterization of the disease has been made in comparison with the results of neuropathological study of 64 cases. The geographical distribution and epidemiological features of the disease, based on verified clinical material, were also studied. VE is hypothesized to be an infectious disease with a strong inflammatory component, probably slow virus infection. Further studies on neuropathological and etiological aspects of VE have been initiated.

#### Hantaviruses and Hemorrhagic Fever with Renal Syndrome

We were first to demonstrate the presence of a hantavirus (Prospect Hill virus) in meadow voles, an indigenous arvicolid rodent, to demonstrate that the massive Chinese epidemics of HFRS occurring during the past two decades were caused by viruses closely related to viruses isolated in Korea and Siberia, and to prove that two serotypes of hantaviruses were responsible for severe and mild forms of HFRS in Yugoslavia.

A severe, fatal hemorrhagic illnesses with renal insufficiency, of suspected hantavirus etiology in an illegal immigrant in Texas prompted us to investigate the prevalence of hantavirus infection in wild rodents in designated regions in Texas. A virus antigenically distinct from other known hantaviruses has been isolated from Mus musculus captured in Leakey, Texas. Studies are in progress to further characterize this isolate, to determine its role in human infection and disease, and to clarify if the seroepidemiology of Leakey virus infection in feral mice resembles that of Seoul virus infection in urban rats.

We are also continuing to study the mild nephropathy produced by Prospect Hill and Puumala viruses in cynomolgus monkeys. Tests for glomerular filtration (endogenous creatinine and phenolsulfonphthalein clearances) have been normal but proteinuria suggests an impairment of glomerular vascular integrity. Serial renal biopsies are being planned in

infected monkeys. In addition, blood and urine specimens are being tested for viral sequences by the PCR method.

Finally, we are continuing to elucidate the epizootiology of hantaviruse infections in the United States concentrating on determining what animals other than rodents are important in the maintenance of the enzootic cycle. Specifically, predatory small mammals (such as weasels and shrews) and birds (such as hawks and owls) are being tested for evidence of hantavirus infection, and virus isolation attempts are being planned from seropositive animals.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01282-25 CNSS

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Population Isolates. Study of Child Growth, Development, Behavior and Learning, and Disease Patterns in Isolated and Primitive Groups

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                               |                           |       |
|---------|-------------------------------|---------------------------|-------|
| PI:     | D. C. Gajdusek, M.D.          | Chief                     | LCNSS |
| Others: | Clarence J. Gibbs, Jr., Ph.D. | Deputy Chief              | LCNSS |
|         | David M. Asher, M.D.          | Research Medical Officer  | LCNSS |
|         | Paul Brown, M.D.              | Medical Director          | LCNSS |
|         | Ralph M. Garruto, Ph.D.       | Senior Research Biologist | LCNSS |
|         | Richard Yanagihara, M.D.      | Medical Director          | LCNSS |

## COOPERATING UNITS (if any)

See Sub-Project Summaries

## LAB/BRANCH

Laboratory of Central Nervous System Studies, Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

12

## PROFESSIONAL:

8

## OTHER:

4

## CHECK APPROPRIATE BOX(ES)

|  |   |                                      |
|--|---|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input checked="" type="checkbox"/> (a1) Minors        |   |                                      |
| <input checked="" type="checkbox"/> (a2) Interviews    |   |                                      |

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies of human biology of vanishing primitive societies focus on neurological development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which most of our studies evolved: kuru-CJD, HIV (AIDS), HTLV-I slow virus infections of the CNS, aging and Alzheimer's disease, dementia, ALS/PD. Techniques of molecular genetics, biochemistry, immunology, virology, and field epidemiological, clinical, linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens from expeditions to Micronesia, Melanesia, Polynesia, South America, Asia and Africa proved valuable in recent HIV (AIDS), HTLV-I, Hantavirus, JC virus of PML and herpesvirus, CMV and EBV studies. Studies on nutrition, reproduction, fertility, age of puberty and aging, genetic distance and pleomorphisms, unusual and odd use of the higher cortical functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, HTLV-I myelopathy, epilepsy, familial parkinsonism, Villiisk encephalopathy, other CNS degenerations, hysterical disorders, schizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections in these isolated groups have yielded widely significant discoveries. HFRS caused by Hantaviruses in Asia, USSR, Europe and newly recognized Hantaviruses in the U.S. are studied. Human evolution and adaptability to high altitude, wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychological stress are studied in appropriate population isolates. Thus, HTLV-I and HIV retroviruses causing CNS disease in man we first found in isolated and segregated groups; the variable clinical and epidemiological patterns of both AIDS and HTLV-I are best studied in these diverse isolated groups.



## **I. Mechanisms of dissemination and transmission of HTLV-I in the Caribbean basin, South America and Melanesia**

PI: Ralph M. Garruto, Ph.D. Senior Research Biologist

|                              |                      |
|------------------------------|----------------------|
| Pamela Rodgers-Johnson, M.D. | Visiting Scientist   |
| Carlos Mora, M.D.            | Visiting Associate   |
| Richard Yanagihara, M.D.     | Medical Director     |
| Marta Monzon, Ph.D.          | Visiting Scientist   |
| Mark Miller, B.A.            | Howard Hughes Fellow |

### **Cooperating Units:**

Owen Morgan, University of West Indies, Kingston, Jamaica; Vladimir Zaninovic, Universidad del Valle, Cali, Colombia; Luis Cartier-Roviroso, Universidad de Chile, Santiago, Chile; Carol L. Jenkins, Institute of Medical Research, Goroka, Papua New Guinea; Andrew Ajdukiewicz, Central Hospital, Honiara, Solomon Islands; Steve Alexander, Biotech Research Laboratories, Rockville, Maryland

We were first to demonstrate high titers of IgG antibodies against HTLV-I in cerebrospinal fluids from patients in Jamaica and the Pacific coast of Colombia with Jamaican neuropathy and Pacific spastic paraparesis, respectively. We have now identified cases of HTLV-I-caused spastic paraparesis along the Caribbean coast of Colombia, as well as among Caucasians and non-black Mestizos living in Santiago, Chile, a city in a temperate zone, and in a life-long resident of Honiara in the Solomon Islands. Our studies of more than 3000 sera, collected between 1956 and 1988 from 31 Melanesian populations, for antibodies against HTLV-I, indicate that HTLV-I infection, as verified by strict Western analysis criteria, is as prevalent in several Melanesian communities as in HTLV-I-endemic regions, such as southwestern Japan and the Caribbean basin. Furthermore our studies of families among remote New Guinean groups, such as the Hagahai in Madang Province, indicate that, as in other HTLV-I-endemic areas, HTLV-I seroprevalence is higher in families in which the mother is infected. We are continuing to investigate the basis of the high frequency of indeterminate Western immunoblots in New Guinea to determine if it results from the circulation of novel retroviruses.

## **II. Epidemiology of Creutzfeldt-Jakob disease in recipients of pituitary gland-derived human growth hormone**

PI: Paul Brown, M.D. Medical Director

|                               |  |
|-------------------------------|--|
| Lev Goldfarb, M.D.            | Visiting Scientist                     |
| Clarence J. Gibbs, Jr., Ph.D. | Research Microbiologist (Deputy Chief) |

### **Cooperating Units:**

Judith Fradkin, NIDDK, DDEM, Bethesda

Continuing surveillance of the outbreak of Creutzfeldt-Jakob disease (CJD) in young people treated with pituitary gland-derived human growth hormone (hGH) has to date identified 11 subjects (of whom seven were treated in this country) dying of CJD between 4 and 20 years after their last dose of hormone. It now appears that, world-wide, the risk of developing CJD in hGH-treated patients is at least 1 per thousand, and possibly as high as 1 per hundred, compared to the risk of 1 per million in the general population. Extensive analysis of processing records implicates multiple batches of pituitary glands as the source of contamination, and all patients so far identified have been treated before 1976. Molecular genetic analysis of DNA from two patients has raised the possibility of a mutation in the scrapie amyloid gene as a susceptibility factor in determining which patients among the treated population develop disease.

Additional cases of iatrogenic CJD due to treatment with pituitary gland-derived gonadotropin and to implantation of dura matter grafts have also been identified, and consultations with the FDA have assisted in revised policy decisions.

### III. Elucidation of the cause and pathogenesis of high-incidence motor neuron disease in different climatic regions and among diverse ethnic groups

Co-PI: Ralph M. Garruto, Ph.D.      Senior Research Biologist  
Richard Yanagihara, M.D.      Medical Director

Michael J. Strong, M.D.      Guest Researcher  
Don C. Guiroy, M.D.      Visiting Associate  
Pamela Rodgers-Johnson, M.D.      Visiting Scientist  
Pedro Piccardo, M.D.      Visiting Associate

#### Cooperating Units:

Kwang-Ming Chen, Olivia Cruz, Guam Memorial Hospital, Agana, Guam; Chris C. Plato, NIA, Gerontology Research Center, Baltimore, Maryland

Our multidisciplinary approach to the study of high-incidence motor neuron disease, conducted during the past three decades among geographically and genetically diverse groups in the Western Pacific, indicates unequivocally that there is no genetic cause, but rather a defect in mineral metabolism, provoked by chronic nutritional deficiencies of calcium, leads to increased intestinal absorption of toxic metals and the intraneuronal co-deposition of calcium, aluminum and silicon. This elemental deposition interferes with slow axonal transport by altering neurofilament production and/or catabolism, resulting in excessive neurofilament accumulation. Studies are in progress to sequence the amyloid isolated from brains of Guamanians with amyotrophic lateral sclerosis and from tissues of neurologically normal Guamanians with neurofibrillary pathology to determine if it differs from the amino acid sequence of neurofibrillary tangles isolated from Guamanians with parkinsonism-dementia. We are also investigating the cause of the frequent occurrence of neurofibrillary pathology in neurologically normal Guamanians because of the obvious implications to normal neuronal aging. Furthermore, epidemiological investigations of the development of motor neuron disease among migrants who left the high-incidence foci during infancy or childhood are planned.

### IV. Worldwide epizootiology and epidemiology of hantavirus infection: search for human disease in the face of a widespread enzootic in the United States

PI: Richard Yanagihara, M.D.      Medical Director

David M. Asher, M.D.      Research Medical Officer  
Bruce Johnson, Ph.D.      Special Expert  
Shuyuan Xiao, M.D.      Visiting Fellow  
Zayd Eldadah      Biological Lab Aid  
Clarence J. Gibbs, Jr., Ph.D.      Research Microbiologist (Deputy Chief)

#### Cooperating Units:

Theodore F. Tsai, CDC, Ft. Collins, Colorado; Patrick Redig, Raptor Center, St. Paul, Minnesota; Duane Schlitter, Carnegie Museum of Natural History, Pittsburg, Pennsylvania; Robert Traub, Smithsonian Institution, Washington, D.C.; Ana Gligic, Institute of Virology, Belgrade, Yugoslavia; Yong Kang, University of Ottawa, Ottawa, Canada; Chin-Ming Hsiang, Hubei Medical College, Hubei, People's Republic of China

We were first to isolate hantaviruses from meadow voles (*Microtus pennsylvanicus*) and mice (*Mus muscalus*) captured in the United States, to demonstrate that the massive epidemics of hemorrhagic fever with renal syndrome (HFRS) in the People's Republic of China were caused by viruses closely related to hantaviruses isolated in Korea and Siberia, and to prove that two serotypes of hantaviruses were responsible for severe and mild forms of HFRS in Yugoslavia. Despite the widespread distribution of hantaviruses in commensal rats and indigenous wild rodents in the United States, confirmed cases of HFRS have not been recognized and the overall risk of hantavirus infection in Americans is low, even among individuals who have frequent exposure to wild rodents. In an effort to further clarify the epizootiology, ecology and epidemiology of hantavirus infection in the United States, we are currently examining sera from predatory small mammals (particularly, shrews and weasels) and birds (such as owls and hawks), as well as sera from patients with non-A, non-B hepatitis and from muskrat trappers for evidence of hantavirus infection. We are also investigating the distribution of these viruses among indigenous arvicolid and cricetid rodents in South America and Canada, and the epizootiology of Leakey virus infection in *Mus* populations.

#### V. Studies of high-incidence non-neurological disorders in specific racial and ethnic groups

|     |                          |                           |
|-----|--------------------------|---------------------------|
| PI: | Ralph M. Garruto, Ph.D.  | Senior Research Biologist |
|     | Mark A. Miller, B.A.     | Howard Hughes Fellow      |
|     | Richard Yanagihara, M.D. | Medical Director          |

#### Cooperating Units:

Julianne Imperato-McGinley and Ralph Peterson, Cornell University Medical College, New York, New York; Charles Weitz, Temple University, Philadelphia, Pennsylvania; Chen-ting Chin, Beijing University Medical School, Beijing, People's Republic of China

For 25 years, we have studied a focus of male pseudohermaphroditism among small, remote, inbred, forest-dwelling, hoe and digging-stick horticulturalists of the Simbari Anga linguistic group of the Eastern Highlands of Papua New Guinea. Clinically, these male pseudohermaphrodites represent a spectrum of congenital anatomical abnormalities including a foreskin forming a small fold above a rudimentary clitoris-like penis and bilateral scrotal flaps resembling labia enclosing small testes. There is a urogenital sinus containing a urethra and a blind vaginal pouch. Patients show no gynecomastia or menses. At puberty, the clitoris-like penis and testes enlarge with concurrent extensive facial, pubic and axillary hair growth and musculature development greater than in their normal male peers. Sera collected from two of these young adult patients revealed elevated testosterone/dihydrotestosterone ratios. Both had high urinary etiocholanolone/ androsterone, C19 and C21 5 $\beta$ -5 $\alpha$  metabolite ratios. The data indicate a 5  $\alpha$ -reductase deficiency similar to patients studied in the Dominican Republic.

Human adaptation to high altitude has also been intensively investigated. After more than five years of preparation, a collaborative study of minority groups living at high altitude in Qinghai Province of the People's Republic of China will be implemented.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00969-25 CNSS

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                               |                           |       |
|---------|-------------------------------|---------------------------|-------|
| PI:     | D.C. Gajdusek, M.D.           | Chief                     | LCNSS |
| Others: | Clarence J. Gibbs, Jr., Ph.D. | Deputy Chief              | LCNSS |
|         | David M. Asher, M.D.          | Research Medical Officer  | LCNSS |
|         | Paul Brown, M.D.              | Medical Director          | LCNSS |
|         | Ralph M. Garruto, Ph.D.       | Senior Research Biologist | LCNSS |
|         | Richard Yanagihara, M.D.      | Medical Director          | LCNSS |
|         | (continued see next page)     |                           |       |

## COOPERATING UNITS (if any)

See Sub-Project Summaries

## LAB/BRANCH

Laboratory of Central Nervous System Studies, Basic Neurosciences Program, Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

22

## PROFESSIONAL:

18

## OTHER:

4

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies focus on causes and pathogenesis of chronic degenerative CNS disorders with emphasis on MS; Parkinson's, Pick's, Huntington's and Alzheimer's diseases; ALS/PD of Western Pacific; supranuclear palsy; other presenile dementias; spinocerebellar ataxias; epilepsy; chronic encephalitis with focal epilepsy; Viliuisk encephalopathy; muscular dystrophies; chronic schizophrenia; autism; SSPE; PML; dialysis encephalopathy; goiterous cretinism; cysticercosis; and intracranial neoplasm.

We have defined the transmissible and nontransmissible dementias as cerebral amyloidoses caused by post-translational modification of a specific host precursor protein to amyloid fibril deposits. We now recognize the slow unconventional viruses causing kuru-CJD-scrapie as replicating polypeptides formed *de novo* from a normal host precursor protein, specified on chromosome 20 in man and 2 in mice. The molecular elucidation of the spontaneous configurational change to infectivity, basically a crystallographic problem, is now becoming our major target. Molecular genetic analysis of familial CJD already indicates several point mutations which enormously increase ( $\times 10^6$ ) the probability of this spontaneous *de novo* conversion to an infectious polypeptide. Microbiology must now contend with a totally new paradigm for replicating, infectious, pathogenic agents in the nontransmissible brain amyloidoses. Our studies focus on the elucidation of the molecular configurational events conferring the property of infectivity on a previously normal host precursor.

In normal aging, Alzheimer's disease (AD), and Down's syndrome a different host precursor protein (specified on chromosome 21 in man, 16 in mice) is a cell excreted inhibitor of growth factors. Post-translational degradation of this normal precursor forms the 42 amino acid amyloid polypeptide which polymerizes to form the deposits of amyloid angiopathy, amyloid plaques and neurofibrillary tangles in aging, AD and Down's. This occurs in all individuals who reach their 90s. Genetic, toxic, and infectious factors may accelerate this aging brain amyloid deposition.

Conventional viruses causing slow, infectious, degenerative disease are intensely studied to elucidate the neurotropism and mechanism of pathogenesis: retrovirus encephalomyelopathies of HTLV-I and HIV (of AIDS); herpesviruses (HSV, CMV, EB and virus varicella-zoster); papovaviruses (JC); RSSE; measles; SSPE; and many chronic virus infections of amyloid..



## I. Molecular pathogenesis of the transmissible cerebral amyloidoses

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|-------------------------------|--|
| Co-PI: Paul Brown, M.D.       | Medical Director                       |
| Clarence J. Gibbs, Jr., Ph.D. | Research Microbiologist (Deputy Chief) |
| Jiri Safar, M.D.              | Visiting Fellow                        |
| Mauro Ceroni, M.D.            | Visiting Associate                     |
| Pedro Piccardo, M.D.          | Visiting Associate                     |
| Don C. Guiryo, M.D.           | Visiting Associate                     |
| Pawel P. Liberski, M.D.       | Visiting Fellow                        |

Our work indicates that the scrapie amyloid precursor protein is converted into an infectious form by configurational changes of the normal precursor. In susceptible hosts, the scrapie monomer may act much like the fibril amyloid-enhancing factors found in AA amyloidosis to autonucleate and autopattern this conversion, resulting in its own polymerization or crystallization and precipitation as insoluble arrays of amyloid fibrils. Using tissues stored from experimentally infected animals, we have begun to determine the structure and sequence of the amyloid protein of different strains of virus recovered from the same genetic host. In addition, we have determined the intracellular localization of the amyloid precursor protein, and are now trying to define the pathway of scrapie amyloid formation and to interrupt its formation *in vivo*.

## II. Molecular genetics of the PRIP gene in the subacute spongiform virus encephalopathies

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|-------------------------|--------------------------|
| Co-PI: Paul Brown, M.D. | Medical Director         |
| Lev Goldfarb, M.D.      | Visiting Scientist       |
| David M. Asher, M.D.    | Research Medical Officer |
| Pedro Piccardo, M.D.    | Visiting Associate       |

### Cooperating Units:

Dmitry Goldgaber, State University of New York, Stonybrook, New York

We have demonstrated several mutations in the open reading frame of the gene encoding the scrapie precursor protein which seem to be linked to the human transmissible encephalopathies. An amino acid-altering mutation in codon 102 was identified in three patients with Gerstmann-Straussler syndrome, adding a large and well documented family of German origin to two previously reported unrelated American and English families. Brain tissue from one of these patients has transmitted the disease to experimental animals. This mutation was not found in 8 unaffected family members, 25 healthy control individuals, 3 transmitted cases of kuru, 17 transmitted cases of Creutzfeldt-Jakob disease (4 familial and 13 sporadic), and 11 patients with other neurological disorders.

A different double-allele mutation in codon 129 was found in 2 of 3 kuru patients, 3 of 3 patients with familial CJD belonging to three related families, and 2 of 2 patients with iatrogenic CJD caused by treatment with contaminated pituitary-derived human growth hormone. The nucleotide change in this codon (ATG to GTG) resulted in an amino-acid substitution of valine for methionine; it also abolished an Nsp1 restriction site and created a new MaeII site, which permitted the use of a restriction-endonuclease technique as a screening procedure for this mutation.

Using direct sequencing and restriction-endonuclease analysis in additional cases, we failed to find this double-allele mutation in patients with sporadic CJD or in healthy controls, but an identical single-allele codon 129 mutation (heterozygosity) was found in 3 of 15 patients with sporadic CJD and in 3 of 24 healthy control individuals. Detection of a consistent double-allele mutation in 7 studied patients with related disorders suggests that homozygosity for a PRIP-129 point mutation may serve as the

genetic background for some cases of kuru and CJD. It is likely that other amino acid-altering mutations in the PRIP gene will be found in patients with sporadic or familial CJD.

### III. *In vitro* and *in vivo* models of ALS pathogenesis

PI: Ralph M. Garruto, Ph.D. Senior Research Biologist

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|--------------------------|-----------------------|
| Richard Yanagihara, M.D. | Medical Director      |
| Michael J. Strong, M.D.  | Guest Researcher      |
| Axel V. Wolff, D.V.M.    | Facility Veterinarian |

#### Cooperating Units:

Charles E. Fiori, BEIB, NIH, Bethesda; Andres Salazar, Walter Reed Army Medical Center, Washington, D.C.; S.M. Chou, Case Western Reserve University, Cleveland, Ohio

To test the hypothesis that chronic dietary deficiencies of calcium and magnesium is involved in the pathogenesis of amyotrophic lateral sclerosis (ALS), we maintained young cynomolgus monkeys for nearly four years on a diet low in calcium and magnesium and with or without supplemental aluminum and unwashed cycad. Calcium-deprived monkeys developed unequivocal motor neuron pathology, consisting of aberrant perikaryal accumulation of phosphorylated neurofilaments and axonal spheroids, which resemble that seen in ALS. *In vitro* studies of fetal mouse motor neuron cultures exposed to micromolar concentrations of aluminum chloride, and *in vivo* studies in rabbits injected intracisternally with microgram quantities of aluminum salts, showed similar abnormal ALS-like accumulations of neurofilament. We have also been experimenting with other toxins which produce neurofilamentous pathology in animals and fetal motor neuron cultures, and are systematically investigating neurofilament metabolism and catabolism in these systems.

### IV. Cell biology and molecular pathogenesis of deposition of aging brain amyloid

PI: Ralph M. Garruto, Ph.D. Senior Research Biologist

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|--------------------------|--------------------|
| Michael J. Strong, M.D.  | Guest Researcher   |
| Don C. Guiray, M.D.      | Visiting Associate |
| Richard Yanagihara, M.D. | Medical Director   |
| Arne Svedmyr, M.D.       | Visiting Scientist |
| Lev Goldfarb, M.D.       | Visiting Scientist |

#### Cooperating Units:

Dmitry Goldgaber, State University of New York, Stonybrook, New York; Ryo Fukatsu, Sapporo Medical College, Sapporo, Japan

Our original cloning and chromosomal localization of the gene encoding the aging brain amyloid precursor protein ( $\beta$ -protein; A4 protein) was followed rapidly by the demonstration that  $\beta$ -amyloid mRNA expression is elevated in neurons which develop neurofibrillary tangles, and has led to the realization that this gene is identical to that encoding an excreted, rapidly turned-over protein which specifically binds to the  $\gamma$ -subunit of nerve growth factor and many other serine proteases. We also know now that  $\beta$ -amyloid mRNA expression in fetal rabbit hippocampal neurons is developmentally regulated and can be modulated in endothelial cell cultures by regulatory molecules such as interleukin-1. Studies are underway to better define the mechanisms of amyloid deposition in Alzheimer disease and parkinsonism-dementia of Guam and to determine if  $\beta$ -amyloid acts as a neurotoxin.

## V. Inactivation studies of the transmissible spongiform encephalopathy agents

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|-------------------------|--------------------------|
| PI: Paul Brown, M.D.    | Medical Director         |
| Pawel P. Liberski, M.D. | Visiting Fellow          |
| David M. Asher, M.D.    | Research Medical Officer |
| Axel V. Wolff, D.V.M.   | Facility Veterinarian    |
| Jiri Safar, M.D.        | Visiting Fellow          |

Ongoing studies of the resistance of scrapie and Creutzfeldt-Jakob disease (CJD) viruses have had both practical and theoretical goals. At the practical level, methods have been devised for general decontamination of the workplace and materials used by hospital and laboratory personnel, and a technique has been discovered that sterilizes tissues for histopathological processing, while maintaining histological integrity. At the theoretical level, experiments using combination treatments with formaldehyde and autoclaving, dry heat up to temperatures of 360°C, sequential enzyme digestions, and polyacrylamide gel electrophoresis have established the protective effect of formaldehyde upon heat inactivation, the survival of small amounts of infectivity even at 360°C, the primacy of fibrillary amyloid protein for virus replication, even stripped of its post-translationally modified non-peptide components.

Further, scrapie-infected brain homogenates, buried and left under natural climatic conditions for a 3-year period, are now under titration to determine the ability of the virus to survive. Survival of the agent will have profound implications for the epidemiology of the disease.

## VI. *In vivo* and *in vitro* studies to detect the etiological agent of Creutzfeldt-Jakob disease in pituitary gland-derived human growth hormone

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| PI: Clarence J. Gibbs, Jr., Ph.D. | Research Microbiologist (Deputy Chief) |
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### Cooperating Units:

Judith Fradkin, NIDDK, DDEM, Bethesda

More than 80 lots of pituitary gland-derived human growth hormone (hGH), some of which were prescribed to young people who subsequently developed Creutzfeldt-Jakob disease (CJD), are being analyzed in *in vivo* and *in vitro* studies designed to detect the etiological agent of CJD. Aliquots of each lot of hormone have been injected into two squirrel monkeys each on two separate occasions and several pooled lots have been injected into chimpanzees to assay for infectivity. Additionally, each lot of hGH and sera from more than 300 recipients of one or more of the lots under study, are being analyzed by enzyme immunoassay, SDS-PAGE and Western analysis. To date, 29 squirrel monkeys have died of intercurrent infections over a 4-year period and the remaining animals are asymptomatic. Antibodies against CJD amyloid-associated protein (PrP<sub>27-30</sub>) have not been detected in the sera of hGH recipients. By SDS-PAGE and Western immunoblot, both pituitary gland-derived and synthetic recombinant types of hGH contain proteins in the same molecular weight range as that of PrP<sub>27-30</sub>.

## VII. Bovine spongiform encephalopathy: experimental transmission of scrapie to three breeds of cattle

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| PI: Clarence J. Gibbs, Jr., Ph.D. | Research Microbiologist (Deputy Chief) |
| Jiri Safar, M.D.                  | Visiting Fellow                        |
| Mauro Ceroni, M.D.                | Visiting Associate                     |
| Alessandro di Martino             | Guest Researcher                       |



## Cooperating Units:

J. Hourrigan and W. Clarke, U.S. Department of Agriculture, Mission Field Station, Mission, Texas

In 1976, investigators at the Scrapie Field Station in Mission, Texas, inoculated five cattle each with a Suffolk sheep strain and an Angora goat strain of scrapie. One animal, a mixed breed of Jersey and Hereford, inoculated with the Suffolk sheep scrapie strain, and two animals, a Jersey and a Hereford, inoculated with the Angora goat strain, developed a progressive neurological disease 37, 27 and 36 months, respectively, following inoculation. Histopathological findings of brains obtained at autopsy were reportedly not confirmatory of scrapie. Recently, we have had the opportunity of re-examining the brains of all 10 inoculated cattle for the scrapie amyloid-associated protein (PrP<sub>27-30</sub>), and have successfully detected this protein in the brains of the three cattle that developed neurological disease. Attempts are underway to transmit the cattle disease to mice, hamsters and additional cattle. This year, in collaboration with the AIREN Foundation, NINDS convened an International Roundtable on bovine spongiform encephalopathy under the chairmanship of the principal investigator of this study.

### VIII. Ultrastructural pathology of the subacute spongiform encephalopathies: serial and comparative studies

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| Co-PI: David M. Asher, M.D. | Research Medical Officer |
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| Pedro Piccardo, M.D.        | Visiting Associate       |
| Shuyuan Xiao, M.D.          | Visiting Fellow          |
| Kitty L. Pomeroy, B.S.      | Microbiologist           |
| Paul Brown, M.D.            | Medical Director         |

## Cooperating Units:

G.A.H. Wells, Ministry of Agriculture, Fisheries and Food, Surrey, United Kingdom; Harash Narang, General Hospital, Newcastle-upon-Tyne, United Kingdom

Parallel arrays of tubulovesicular structures, measuring 20 to 50 nm in diameter, have been consistently observed in both post-synaptic (dendrites) and presynaptic processes of animals with experimentally induced spongiform encephalopathies, irrespective of the host species. We have recently demonstrated these structures in the brain of a Fresian/Holstein cow with bovine spongiform encephalopathy from England. These structures appear before the onset of clinical disease and of other neuropathological changes in mice and hamsters infected with CJD virus and scrapie virus, respectively. To further characterize these structures, ultramicrocryotome-cut sections of brains from scrapie-infected hamsters are being examined by immune electron microscopy.

We have also found that neuroaxonal dystrophy is a prominent feature of the spongiform encephalopathies, and myelin sheath dilatation is a constant finding. This latter finding is indistinguishable from that induced in spinal cord cultures by recombinant human tumor necrosis factor (TNF). Our recent localization of TNF-laden hypertrophic astrocytes in anatomical regions with striking myelin dilatation suggests that TNF may be involved in myelin vacuolation in the subacute spongiform encephalopathies. Studies to quantitate TNF mRNA in brains of CJD virus-infected mice are in progress.

Finally, attempts are being made to characterize the factor present in homogenates of immature rat cerebellar cortex (prepared from 10-day old BD-IX rats when synaptogenesis is at its peak), which

reportedly produces lesions akin to the transmissible spongiform encephalopathies, such as the multilamellated membranes seen in spider monkeys experimentally infected with kuru.

#### IX. Attempts to produce neuro-AIDS in experimental animals with human immunodeficiency viruses

PI: Clarence J. Gibbs, Jr., Ph.D. Research Microbiologist (Deputy Chief)

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|-----------------------|--------------------------|
| David M. Asher, M.D.  | Research Medical Officer |
| Bruce Johnson, Ph.D.  | Special Expert           |
| Hiroko Minagawa, M.D. | Visiting Fellow          |
| Mark A. Beilke, M.D.  | Medical Staff Fellow     |
| Gary Stone, M.S.      | Biologist                |
| Maneth Gravell, Ph.D. | Research Microbiologist  |

#### Cooperating Units:

Prem Sarin, NCI, LTCB, Bethesda; Jaap Goudsmit, University of Amsterdam, Amsterdam, Netherlands

In an effort to develop an animal model of neuro-AIDS, we have inoculated multiple species of New and Old world monkeys, chimpanzees, domestic horses, goats and small laboratory rodents with autopsy tissues, whole blood or plasma from patients with AIDS or pre-AIDS, as well as with supernatant fluids from cell cultures infected *in vitro* with different strains of HIV-1. Although chimpanzees were readily susceptible to infection, and while some continue to remain viremic for more than five years, none has developed clinical AIDS. By contrast, all other nonhuman primate species and other experimental animals were rather resistant to infection, and to date, only one rhesus monkey, one cynomolgus monkey and six horses have shown serological evidence of subclinical infection.

#### X. Virological and molecular genetics studies on simian immunodeficiency viruses as a model for AIDS

PI: Maneth Gravell, Ph.D. Research Microbiologist

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|-------------------------------|--|
| Clarence J. Gibbs, Jr., Ph.D. | Research Microbiologist (Deputy Chief) |
| Mark A. Beilke, M.D.          | Medical Staff Fellow                   |
| Gary Stone, M.S.              | Biologist                              |
| Elaine Kay Jordan, D.V.M.     | Senior Staff Fellow                    |
| Marta Monzon, Ph.D.           | Visiting Scientist                     |
| Rebecca Hamilton              | Microbiologist                         |

#### Cooperating Units:

P.R. Johnson, Georgetown University, Washington, D.C.

In an effort to develop an animal model of neuro-AIDS, we have inoculated multiple species of Old World monkeys with simian retroviruses and both rhesus and pigtailed macaques with strains of simian immunodeficiency virus (SIV) isolated from African green and sooty mangabey monkeys. Three of six rhesus monkeys inoculated with an SIV isolate from a sooty mangabey monkey have developed an AIDS-like disease with impaired motor function and orofacial dyskinesia. Enlarged lateral ventricles and defects in the cerebral cortex were found by MRI brain scan in one of these monkeys one week before death. Vascular gliosis and neuronal loss were evident, particularly in the brain stem, and virus was recovered from brain, spinal cord, cerebrospinal fluid, peripheral nerve and muscle. Further studies are underway to clarify the pathogenesis of AIDS encephalopathy and dementia.

## **XI. Prevention of AIDS: antigenic potency and immunogenicity of inactivated HIV vaccine and synthetic HIV core polypeptide immunogen**

PI: Clarence J. Gibbs, Jr., Ph.D. Research Microbiologist (Deputy Chief)

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|-----------------------|-------------------------|
| Maneth Gravell, Ph.D. | Research Microbiologist |
| Bruce Johnson, Ph.D.  | Special Expert          |
| Hiroko Minagawa, M.D. | Visiting Fellow         |
| Carlos Mora, M.D.     | Visiting Associate      |
| Gary Stone, M.S.      | Biologist               |

### **Cooperating Units:**

Jonas Salk, Salk Institute, La Jolla, California; Frederick Jensen, Immune Response Corporation, La Jolla, California; Prem Sarin, NCI, LTCB, Bethesda; Allen Goldstein, George Washington University, Washington, D.C.

Following repeated doses of an inactivated whole virus HIV vaccine devoid of *env* gp 120/160, chimpanzees persistently infected with HIV developed non-anamnestic antibody responses and were cleared of viremia for one year following virus challenge, beginning at 10 weeks following primary vaccination, as evidenced by negative virus isolation attempts and inability to detect HIV DNA sequences by polymerase chain reaction. Additional safety, toxicity and immunogenicity studies are underway using a synthetic 30-amino acid peptide of a conserved p17 region of HIV-1. Studies are in progress to assess the utility of each of these vaccines to prevent AIDS in humans already infected with HIV.

## **XII. Antigenic, virological and molecular biological characterization of HTLV-I strains circulating in high incidence in the Caribbean basin, South America and Melanesia**

Co-PI: Richard Yanagihara, M.D. Medical Director  
Clarence J. Gibbs, Jr., Ph.D. Research Microbiologist (Deputy Chief)

|                         |                           |
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| Ralph M. Garruto, Ph.D. | Senior Research Biologist |
| Carlos Mora, M.D.       | Visiting Associate        |
| David M. Asher, M.D.    | Research Medical Officer  |
| Marta Monzon, Ph.D.     | Visiting Scientist        |
| Pawel P. Liberski, M.D. | Visiting Fellow           |
| Mark Miller, B.A.       | Howard Hughes Fellow      |

### **Cooperating Units:**

Prem Sarin, NCI, LTCB, Bethesda; Michael P. Alpers, Institute of Medical Research, Goroka, Papua New Guinea; Jaap Goudsmit, University of Amsterdam, Amsterdam, Netherlands; Steve Alexander, Biotech Research Laboratories, Rockville, Maryland

Multiple HTLV-I isolates have been made from peripheral blood lymphocytes and cerebrospinal fluid cells from Jamaican, Colombian and Chilean patients with tropical spastic paraparesis. Molecular genetic analyses of some of these isolates indicate minor differences from strains of HTLV-I isolated from patients with lymphoproliferative malignancy, such as adult T-cell leukemia. Further comparisons between TSP strains of HTLV-I are in progress.

Although we had long established the high prevalence of HTLV-I infection in remote population groups in Melanesia, peripheral blood mononuclear cells from seropositive individuals living in these high-prevalence villages have only recently been made available. We have now visualized by thin-section electron microscopy type C retrovirus particles in T-cell cultures derived from peripheral blood

lymphocytes of New Guineans with confirmatory and indeterminate Western immunoblots, and have isolated an HTLV-I from one of these cultures. Further characterization of this isolate should establish whether or not the high frequency of indeterminate Western immunoblots among Melanesians is a direct result of the existence of closely related but distinct retroviruses.

### **XIII. Experimental HTLV-I infection in nonhuman primates and rabbits: pathogenesis and virus neurotropism**

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|--------------------------------------|--|
| Co-PI: Clarence J. Gibbs, Jr., Ph.D. | Research Microbiologist (Deputy Chief) |
| David M. Asher, M.D.                 | Research Medical Officer               |
| Hiroko Minagawa, M.D.                | Visiting Fellow                        |
| Bruce Johnson, Ph.D.                 | Special Expert                         |
| Carlos Mora, M.D.                    | Visiting Associate                     |
| Maneth Gravell, Ph.D.                | Research Microbiologist                |
| Marta Monzon, Ph.D.                  | Visiting Scientist                     |
| Pawel P. Liberski, M.D.              | Visiting Fellow                        |
| Gary Stone, M.S.                     | Biologist                              |

#### **Cooperating Units:**

Jaap Goudsmit, University of Amsterdam, Amsterdam, Netherlands

In an attempt to establish an animal model of tropical spastic paraparesis (TSP), we have inoculated chimpanzees, African green monkeys and rabbits by various routes, including intraspinally, with different strains of HTLV-I, some of which were isolated from patients with TSP. All animals have seroconverted and remain viremic (for chimpanzees, nearly six years postinoculation), but none have developed disease. Selected regions of several HTLV-I genes, amplified by the polymerase chain reaction, are being sequenced, and compared with the sequences of the original infecting strain, to determine the genetic stability of HTLV-I in persistently infected chimpanzees. DNA extracted from blood of a chimpanzee spontaneously infected with HTLV-I in our colony more than 17 years ago is being similarly studied.

### **XIV. Detection of cryptic viral genomic sequences in tissues from patients with chronic neurological diseases of unknown etiology**

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|--------------------------|---------------------------|
| PI: David M. Asher, M.D. | Research Medical Officer  |
| Richard Yanagihara, M.D. | Medical Director          |
| Mark Godec, M.D.         | NRC Research Fellow       |
| Bruce Johnson, Ph.D.     | Special Expert            |
| Zayd Eldadah             | Biological Lab Aid        |
| Kitty L. Pomeroy, B.S.   | Microbiologist            |
| Michael P. Sulima        | Biological Lab Technician |

#### **Cooperating Units:**

Steven Feinstone, NIAID, LID, Bethesda; William Bellini, CDC, Atlanta, Georgia; Peggy Swoveland, Univ. Maryland, Baltimore, Maryland; Teryl Frei, Georgia State University, Atlanta, Georgia

We have developed a polymerase chain reaction (PCR) technique for detecting genes of several RNA and DNA viruses. While only moderately sensitive compared to classical virus isolation, the technique is able to detect incomplete, defective and degraded viruses in fixed and embedded tissues, as



well as in frozen tissues after decades of storage. The technique is highly specific and is capable of rapidly distinguishing genes of related RNA viruses, such as various related flaviviruses. Even very closely related viruses, like the subtypes of dengue virus, can sometimes be identified by the size of the cDNA product produced by a selected primer pair. The technique is being applied to the amplification of viral RNA and DNA in tissues of patients with chronic neurological disorders of unknown etiology, such as chronic encephalitis, amyotrophic lateral sclerosis, parkinsonism and Viliuisk encephalitis. Thus far, primer pairs have been synthesized for multiple structural genes of several RNA viruses, including measles, mumps, rubella, polio, coxsackie B, HTLV-I, and St. Louis encephalitis viruses, and DNA viruses, including Epstein-Barr virus, herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus. Primer pairs for *Treponema pallidum* and *Toxoplasma gondii* are also being used.

#### XV. Search for an infectious cause of Alzheimer's disease and multiple sclerosis

|     |                      |                          |
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| PI: | David M. Asher, M.D. | Research Medical Officer |
|     | Mark Godec, M.D.     | NRC Research Fellow      |

##### Cooperating Units:

Steven Feinstone, NIAID, LID, Bethesda; Stanley Rapoport and Robert Friedland, NIA, LN, Bethesda

Futher attempts are being made to recover infectious agents from patients with Alzheimer's disease and multiple sclerosis. Buffy coat specimens from more than 100 unaffected family members of patients with familial Alzheimer disease have been inoculated into LVG hamsters to verify the claims, by Manuelidis and his colleagues, of the successful transmission of a spongiform encephalopathy. In addition, CNS tissues, intestinal mucosa and peripheral mononuclear cells from patients with multiple sclerosis are being examined for viral genomic sequences by the polymerase chain reaction technique.

#### XVI. Pathogenesis of hantavirus infection in animals and cell culture

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|--------|--------------------------|--------------------------|
| Co-PI: | David M. Asher, M.D.     | Research Medical Officer |
|        | Richard Yanagihara, M.D. | Medical Director         |
|        | Bruce Johnson, Ph.D.     | Special Expert           |
|        | Shuyuan Xiao, M.D.       | Visiting Fellow          |
|        | Mark Godec, M.D.         | NRC Research Fellow      |
|        | Zayd Eldadah             | Biological Lab Aid       |
|        | Kitty L. Pomeroy, B.S.   | Microbiologist           |
|        | Axel V. Wolff, D.V.M.    | Facility Veterinarian    |

##### Cooperating Units:

David J. Silverman, University of Maryland, Baltimore, Maryland

We have developed and exploited several rodent and primate systems for the study of hantavirus infection, including infant mice and weanling rats infected with Hantaan virus, bank voles (*Clethrionomys glareolus*) infected with Puumala virus and cynomolgus monkeys infected with Puumala and Prospect Hill viruses. We are extending these studies to include the use of the polymerase chain reation (PCR) and renal biopsy in nonhuman primates infected by the parenteral and intranasal routes with disease-causing hantavirus isolates from Yugoslavia and the People's Republic of China. Also, we will analyse blood, urine and postmortem tissues from patients with hemorrhagic fever with renal syndrome for hantaviral sequences by PCR. Furthermore, we are investigating the pathogenesis

of Prospect Hill virus infection in its natural rodent reservoir, the meadow vole. Finally, the mediators released by human vascular endothelial cells experimentally infected with pathogenic strains of hantavirus are being studied.

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CONTRACTS

University of Southwestern Louisiana  
New Iberia Research Center  
New Iberia, Louisiana

Contract #N01-NS-8-00931

\$91,660.00

Program Resources, Inc.  
(Administration by NCI)

Contract #N01-CO-75380

\$420,000.00









# ANNUAL REPORT

October 1, 1988 through September 30, 1989

## Laboratory of Experimental Neuropathology Basic Neurosciences Program, DIR, NINDS

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Experimental Neuropathology, DIR  
Basic Neurosciences Program  
National Institute of Neurological Disorders and Stroke

Henry deF. Webster, Chief

The Laboratory of Experimental Neuropathology (LENP) includes the Cellular Neuropathology Section (CN) and the Neurotoxicology Section (NT). The main goal of the Laboratory's research program is to investigate cellular mechanisms of neurological diseases, especially those that are directly related to acquired immunodeficiency syndrome (AIDS), progressive multifocal leukoencephalopathy (PML), the pathogenesis and latency of herpes simplex virus (HSV) infections, multiple sclerosis, and age-related changes in peripheral nerves. Other closely coordinated projects use biochemical methods to explore gangliosides, protein phosphorylation and lipid peroxidation in the brain. Neurotoxic actions also are investigated.

Important discoveries in LENP's research in AIDS include: (1) In transgenic mice containing proviral HIV that were created by J. Leonard, M. Martin (LMM, NIAID) and associates, LENP in situ hybridization and electron microscope studies showed that high levels of HIV-specific nucleotides were in skin lesions of affected animals and that they were localized in abnormal keratinocytes. 2) In the affected transgenic mice, retarded CNS development was also observed, especially in neuronal populations still migrating postnatally to destinations found in mature animals (i.e. cerebellar granule cells). 3) In human bone marrow cultures infected with HIV-1 by B. Potts (LMM, NIAID), LENP electron microscope and in situ hybridization studies showed that HIV-specific nucleotides were localized in cells of the promonocyte/macrophage lineage. 4) In patients with AIDS the interactions of HIV and herpes viruses are unclear due to imprecise methods for herpes virus identification. An important discovery is antigen detection methodology that permits distinguishing the closely related herpes viruses, HSV-1, HSV-2 and Varicella Zoster virus (VZV) in actively infected human CNS tissues. This methodology will help define HIV-herpes virus interactions that produce CNS lesions. 5) Many AIDS patients develop a demyelinating disease, progressive multifocal leukoencephalopathy (PML), caused by JC virus infection. Lesions characteristic of this disease have been identified for the first time in an African patient with AIDS. Other African cases with both PML and AIDS are being sought.

The most important new findings in other projects are: 1) Studies done in collaboration with E.R. Stadtman (LB, NHLBI) and associates have shown that the concentration of free protein carbonyl groups (an index of protein oxidation) is significantly increased in two experimental models of myelin damage, electrolyte-induced myelinolysis and cuprizone intoxication. These findings have important therapeutic implications for patients with

illnesses that are associated with CNS oxidative stress. (2) An important discovery in studies of cellular metabolism and function is that post-translational covalent modification of protein phosphorylation by gangliosides is observed in many tissues and is not species-specific. For example, in muscle, the effects of gangliosides are mediated in part by stimulation of the intrinsic activity of phosphorylase kinase. Also, gangliosides may regulate glycogen metabolism by enhancing phosphorylation and activating glycogen phosphorylase, a key enzyme for glycogenolysis.

The following summary describes these discoveries and the most significant new evidence obtained in FY 1989 LENP projects.

### Experimental and Human HIV Infections

Transgenic mice containing intact copies of HIV proviral DNA were created by LMM (NIAID) scientists and studied in LENP by in situ hybridization, immunocytochemistry and electron microscopy. Affected mice appeared normal until age 8-10d, when they developed a disease syndrome characterized by retarded growth, epidermal hyperplasia, lymphadenopathy, splenomegaly and pulmonary infiltrates. They died or were sacrificed by 25d of age, and before then they had no other apparent clinical abnormalities. When skin sections were hybridized with HIV-specific probes, the highest signal levels were seen in the lesions and were localized around hair follicles and among individual cells throughout the stratum spinosum (Science, 242:1665-70, 1988). Comparisons of hybridized sections with serially cut sections treated with anti-S-100 and anti-keratin antibodies showed that HIV-specific nucleotides were localized in keratinocytes which also proved to be abnormal when examined electron microscopically. The brains of two affected transgenic mice were examined histologically and no inflammatory lesions, giant cells, perivascular lesions or microglial cell abnormalities were seen. Brain sizes were reduced 22-35%. Hypomyelination, astrocytic hypertrophy and delayed migration of granule cell precursors also were observed. Unfortunately, pituitary function could not be assessed and the loss of this colony in a laboratory accident soon after the sacrifice of these mice precluded further investigation of this developmental defect.

Since the mechanism of the anemia in patients with AIDS is not well understood and a well defined system was needed to evaluate radiolabel distribution found in hybridized transgenic mouse sections, cultured human peripheral blood lymphocytes and bone marrow cells infected with HIV-1 also were fixed and studied in LENP by in situ hybridization and by electron microscopy. High HIV probe labeling was found in PBL mononuclear cell rosettes and on EM examination, all stages of HIV assembly and budding were easily identified. In cultured bone marrow cells, labeling was concentrated in cells of the pro-mononuclear/macrophage lineage.

Initial observations carried out last year in collaboration with Prof. G Gosztonyi, Free University of Berlin suggested that HIV-specific probes might be localized in some neurons and glia in cases of AIDS encephalopathy. To investigate this further, sections containing CNS lesions from 6

patients autopsied at the NIH were selected by Dr. D. Katz (NINDS) and studied by *in situ* hybridization. Abnormally high levels of labeling were not identified in either the neurons or macroglia found in these sections.

### Biochemical and Immunologic Mechanisms in Virally-Induced CNS Demyelination

Understanding of the etiopathogenesis of progressive multifocal leukoencephalopathy (PML) has been hampered in the past by a lack of animal models of this JC virus (JCV) infection of oligodendrocytes and astrocytes. Fortunately, this situation has largely been remedied as three animal models are now available. During this past year we have pursued all three. First, we have extended studies in the neonatal hamster model, in which it has been shown that JCV infection can be detected by T-antigen expression as early as 3 days after injection of the neonate on day 1. The hamster provides a readily accessible model of JCV latency in the CNS and the role of the immune system in controlling the course of the infection, as well as insights into cell specificity. Second, in collaboration with Dr. Om Prakash and Dr. Richard Frisque, transgenic mice have been generated using hybrid polyomavirus early regions in which JCV or SV40 regulatory regions have been combined with the T-antigen coding sequence of the other virus. These hybrids of the closely related human and monkey viruses should provide further insight into the mechanisms of tissue specificity of these viruses, and the mechanisms of tumor induction, as well as of dysmyelination. Third, the macaque is susceptible to a naturally occurring PML due to SV40 virus infection of the CNS. This SV40 infection occurs during both natural and experimental infection of macaques with the simian immunodeficiency virus (SIV). Using material from the California Primate Research Center, we have provided immunocytochemical confirmation of the presence of SV40 in the macaque CNS lesions, and have shown their similarity to lesions occurring in the human disease. While all three models will contribute to our understanding of this unique viral demyelinating disease, clearly the macaque model provides all the elements of the PML/AIDS infection in man, and will therefore assume major importance in the understanding of the pathogenesis and treatment of JCV infection of the CNS.

### Herpesvirus Infection and Nervous System Disease

As an outgrowth of experiments to detect HSV antigen in human autopsy tissues from MS and non-MS tissues, we have performed further tests to distinguish between HSV-1, HSV-2 and VZV antigen in active infections in neural tissues from human autopsy cases. Methods developed permit clear distinction between closely related HSV-1 and HSV-2 antigen and more distantly related VZV antigen.

In a mouse model of intracerebral HSV-2 infection, we have shown that viral transcripts can be detected in latently infected neurons of



trigeminal ganglia, and that the region of the viral genome that is expressed during latency is similar to the latency-associated transcript region of HSV-1.

In a mouse model of genital herpes infection, we have found that peripheral nervous system lesions develop during acute infection. These lesions initially show axonal degeneration, followed by a regenerative response. The regeneration observed has characteristics distinguishing it from classical axotomy models, and for this reason deserves further investigation. Also, this study suggests new ideas on events in the establishment of HSV latency in the neuron.

### Lipid Peroxidation, Protein Oxidation and Demyelination

There are now three animal models where evidence of oxidative stress occurs in association with myelin damage. In the first, treatment with 100% normobaric oxygen after global brain ischemia in the Mongolian gerbil produces significant increases in 14-day cumulative mortality and in brain lipid peroxidation (Mickel HS, et al., Stroke, 1987). Decreased myelin basic protein immunoreactivity and other myelin changes are seen in myelinated fiber bundles located in the lateral corpus striatum, the lateral thalamus and the tegmentum in animals treated with 100% normobaric O<sub>2</sub>. These findings are in contrast with the changes typically seen in ischemic necrosis and a lack of selective myelin lesions, both of which were found in animals treated with air during reperfusion.

In the second model, after correction of severe hyponatremia in rats by treatment with hypertonic saline, a significant increase in oxidation of soluble brain protein was found and myelin lesions typically found in this condition were seen in the corpus striatum and in the thalamus. In animals made hyponatremic, a marked increase in serum iron concentration occurred which decreased quickly upon rapid correction of hyponatremia. Total iron in the brain decreased during hyponatremia, but returned to normal levels by the second day of correction. Total copper in the brain increased during hyponatremia and reached still higher levels by the second day of correction. Further changes in blood chemistries occurring upon rapid correction of hyponatremia were an increase of serum cholesterol to hypercholesterolemic values and an increase in ceruloplasmin levels to above normal. These abnormalities in blood chemistries are further evidence of an oxidative insult and provide a rationale for appropriate treatment of patients with similar findings.

In the third animal model, cuprizone-induced demyelination in the mouse, significant demyelination occurs in the cerebral hemispheres by the fourth week and marked demyelination occurs by the eighth week. Concentration of oxidized soluble brain proteins is increased threefold by the eighth week. Myelin basic protein concentration is reduced by one-half. Levels of oxidized myelin basic protein are going to be determined. After feeding cuprizone, a known copper chelator, at 0.7% of the diet, hepatic copper concentration increased from the first week through the

sixth week, then decreased below normal by the eighth week, with still further decreases seen during recovery.

### Roles of Gangliosides and Protein Phosphorylation in CNS function and Cellular Metabolism.

The major goal of this project is to establish a unified hypothesis that gangliosides can act as multifunctional biomodulators. It is proposed that gangliosides may confer a synchronistic action on modulation of the enzymic activities of several protein kinases which can phosphorylate different target substrates. As a consequence, a synergistic effect may be generated for the regulation of cellular functions. Our previous studies led to the discoveries of two novel ganglioside-dependent protein phosphotransferases in the central nervous system, namely, a ganglioside-stimulated protein kinase and a ganglioside-inhibited protein kinase. In addition, it was found that gangliosides also could regulate the activity of protein kinase C, a  $\text{Ca}^{2+}$ /phospholipid-dependent enzyme ubiquitous in eukaryotic cells. Current research in this laboratory attempts to define the causal relationships between the perturbation of the composition or distribution of gangliosides and the phosphorylation of proteins involved in various metabolic pathways. Results from our investigations thus far indicate that the occurrence of ganglioside-modulated protein phosphorylation systems is widespread. These post-translational covalent modifications systems are not species-specific and could be observed in a number of tissues. In skeletal muscle, gangliosides may regulate glycogen metabolism by enhancing the phosphorylation and activation of glycogen phosphorylase, a key enzyme for glycogenolysis. The effect of gangliosides is mediated, at least in part, through a stimulation of the intrinsic activity of phosphorylase kinase.

Gangliosides also can modulate protein phosphorylation through substrate-directed effects. Interactions between the substrate protein and the glycosphingolipids at or near the sites of phosphorylation may attenuate the phosphorylation processes. Thus, binding of gangliosides to the matrix protein M of vesicular stomatitis virus inhibits the phosphorylation by protein kinase C. Binding of gangliosides to isolated myelin basic protein blocks the phosphorylation of specific sites catalyzed by ganglioside-stimulated protein kinase and by cAMP-dependent protein kinase. Although the exact physiological significance of phosphorylation of myelin basic protein involved in the formation and maintenance of myelin's structure is still unclear, our findings indicate that regulation of this post-translational modification process is complex, especially when gangliosides are present. Further investigations on the inter-relationships among myelin basic protein, gangliosides, and protein phosphorylation may provide better insights into the molecular interactions involved in the maintenance and breakdown of myelin's multilamellar structure.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02549-08 LENP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Herpesvirus Infections and Nervous System Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                 |             |
|---------|----------------------|-----------------|-------------|
| PI:     | J.R. Martin, M.D.    | Medical Officer | LENP, NINDS |
| Others: | S. Suzuki, M.D.      | Visiting Fellow | LENP, NINDS |
|         | D. Henken, Ph.D.     | Staff Fellow    | LENP, NINDS |
|         | H.deF. Webster, M.D. | Chief           | LENP, NINDS |

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Experimental Neuropathology

## SECTION

Cellular Neuropathology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.4

## PROFESSIONAL:

1.4

## OTHER:

1.0

## CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project seeks to define the spectrum of acute, latent and recurrent CNS and PNS disease produced experimentally by herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), to examine the role of HSV-1 and HSV-2 and other herpes virus infections in neurological disease, and to examine the hypothesis that HSV infections may relate to human demyelinative disease, including multiple sclerosis (MS). Our previous studies suggest that several features of HSV epidemiology and pathology may be consistent with an etiological role for HSV infection in MS.

During FY 1989, studies performed show that in human autopsy tissues, active HSV-1, HSV-2 and varicella-zoster virus (VZV) infections can be distinguished using antigen methods. This method may be useful in identification of the infecting agent, particularly when other diagnostic data may be lacking.

In experimental models, in situ hybridization with cDNA probes to HSV-2 genomic sequences have been used to identify neurons that harbor latent HSV-2 infection in trigeminal ganglia following intracerebral inoculation. Further, the region of the HSV-2 genome expressed during latency is similar to that previously found to be expressed in HSV-1 infection. In a model of genital herpes infection, evidence for axonal degeneration and partial regeneration was found. This model may provide new evidence on mechanisms of neural regeneration and HSV latency.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01995-17 LENP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the corners)

Morphological Studies of Myelin Formation, Breakdown and Regeneration

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

|         |                       |                    |             |
|---------|-----------------------|--------------------|-------------|
| PI:     | H. deF. Webster, M.D. | Chief              | LENP, NINDS |
| Others: | L. Lamperth, M.D.     | Visiting Associate | LENP, NINDS |
|         | M.G. Nunzi, Ph.D.     | Guest Researcher   | LENP, NINDS |
|         | K. Tanaka, M.D.       | Visiting Fellow    | LENP, NINDS |
|         | L. Manuelidis, M.D.   | Professor          | Yale Univ.  |

## COOPERATING UNITS (if any)

Department of Functional Morphology, Fidia Research Labs, Italy  
Neuropathology Section, Department of Neurosurgery, Yale Medical School, New Haven, Conn

## LAB/BRANCH

Laboratory of Experimental Neuropathology

## SECTION

Section on Cellular Neuropathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1.00

## PROFESSIONAL

.75

## OTHER

.25

## CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The long range goal of this project is to supplement light and electron microscopic studies with biochemical and morphometric methods to investigate cellular mechanisms of myelin formation, breakdown and regeneration. Current studies and major findings are: (1) to determine the effects of diminished protein synthesis on CNS myelin formation, optic nerves of Xenopus tadpoles were exposed to cycloheximide. Twelve to eighteen hours after immersion, tadpoles were anesthetized, fixed by perfusion, and their optic nerves were examined electron microscopically. After only twelve hours, the numbers of ribosomes in oligodendroglia were greatly reduced, the granular endoplasmic reticulum was disorganized and vesicles filled swollen oligodendroglial tongue processes and paranodal loops at inner margins of developing myelin sheaths. Polyacrilimide gels of cycloheximide-treated optic nerves showed that the treatment had virtually abolished the incorporation of <sup>35</sup>S-labeled methionine into polypeptides. Thus, reduction of protein biosynthesis during CNS myelination has immediate, severe effects on developing sheaths associated with the accumulation of vesicles thought to contain newly synthesized myelin lipids that probably require the presence of proteins for successful incorporation into developing myelin membranes. 2) Other experiments showed that fragments of purified CNS myelin undergo rearrangement to form multilamellar whorls in vitro. A three- to four-fold increase in the number of whorls occurred in 24-hr at 4°C which was time and temperature dependent and occurred without substantial proteolysis or energy input. These observations may help define some of the molecular interactions needed for the creation and maintenance of myelin's compact lamellar structure.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02550-08 LENP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Immunologic Mechanisms in Virally-Induced CNS Demyelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

|     |                        |                                |             |
|-----|------------------------|--------------------------------|-------------|
| PI: | G.L. Stoner, Ph.D.     | Chief, Neurotoxicology Section | LENP, NINDS |
|     | H.deF. Webster, M.D.   | Chief                          | LENP, NINDS |
|     | H. Ressetar, Ph.D.     | Staff Fellow                   | LENP, NINDS |
|     | M. Mazlo, M.D.         | Visiting Scientist             | LENP, NINDS |
|     | C. Ryschkewitsch, B.S. | Medical Technologist           | LENP, NINDS |

## COOPERATING UNITS (if any)

Dept. of Medical Microbiology, Univ. of Wisc. Med. Sch. (D.L. Walker); Dept. of Biochemistry, Pennsylvania State Univ. (R.J. Frisque); Laboratory of Molecular Oncology, Alton Ochsner Medical Foundation, New Orleans (O. Prakash)

## LABORATORY

Laboratory of Experimental Neuropathology

## SECTION

Neurotoxicology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

4.0

## PROFESSIONAL

3.0

## OTHER

1.0

## CHECK APPROPRIATE BOXES)

- |   |   |                                      |
|---|---|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |   |                                      |
| <input type="checkbox"/> (a2) Interviews    |   |                                      |

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

The human polyomavirus known simply as JC virus (JCV) provides the best example of a demyelinating CNS infection because virus replication targets oligodendrocytes and, to a lesser extent, astrocytes. A better understanding of this fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), requires the availability of animal models of the infection. Three animal models have been pursued in this past year. (a) In the first we have further pursued the hamster model in which JCV is inoculated intracerebrally in the neonatal hamster brain and localizes to the cerebellar granular layers, hippocampus, and periventricular areas. Studies of the effect of immunosuppression on the course of this infection, as well as studies on possible transplacental transmission of the virus in the hamster have been initiated. Our findings in the hamster model support our findings of JCV infection in cerebellar granule cells in a case of human PML described previously (Annual Report, 1988), and suggest how low levels of JCV might become focally distributed in the CNS through replication in concert with host cell division. (b) In the second model transgenic mice are being produced in collaboration with Dr. Om Prakash. Hybrid regulatory and coding early regions from JCV and SV40 were used. The first potential founder transgenic mice died of tumors, but lines are now being established which will permit detailed analysis of the effects of incorporation of JCV/SV40 early region into the genome. (c) The third model involves PML due to SV40 in the macaque using material provided by the Primate Research Center, Davis, CA. This model in which spontaneous PML occurs in animals naturally or experimentally infected with SIV very closely parallels PML in human HIV infection (AIDS), and will make a major contribution to our understanding of the pathogenesis and treatment of human PML. Among our studies of human PML is a case originally diagnosed as multiple sclerosis 10 years before. This unusual case appears to combine chronic PML with systemic lupus erythematosus (SLE) of the CNS.

8/LENP/DIR

## NOTICE OF INTRAMURAL RESEARCH PROJECT

201 NS 02699-04 LENP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Roles of Gangliosides in Neuronal and Myelin Function, Cell Growth and Differentiation, and Neurotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

|         |                     |                    |             |
|---------|---------------------|--------------------|-------------|
| PI:     | K.-F.J. Chan, Ph.D. | Sr. Staff Fellow   | LENP, NINDS |
| Others: | Y. Liu, M.D.        | Visiting Fellow    | LENP, NINDS |
|         | S. Komoly, M.D.     | Visiting Scientist | LENP, NINDS |

## COOPERATING UNITS (if any)

None

## LAB. BRANCH

Laboratory of Experimental Neuropathology

## SECTION

Neurotoxicology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.0

## PROFESSIONAL

1.8

## OTHER

0.2

## CHECK APPROPRIATE BOXES:

- |   |   |                                      |
|---|---|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |   |                                      |
| <input type="checkbox"/> (a2) Interviews    |   |                                      |

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The main objective of this project is to establish a unified hypothesis for the biological action of gangliosides as multifunctional biomodulators. Gangliosides are sialic acid-containing glycosphingolipids present in a number of cell types but are particularly abundant in neurons and in myelin. These molecules have been implicated to play important roles in cellular communication such as neurotransmission, neurite outgrowth, synaptogenesis, neuronal regeneration, differentiation, and development. Our studies indicated that gangliosides can act as multifunctional biomodulators. We show that gangliosides have profound effects in modulating protein phosphorylation and dephosphorylation in the central nervous system and in skeletal muscle. The modulatory effects may be mediated through the regulation of the enzymic activities of at least four different protein kinases. These include two novel ganglioside-dependent protein kinases, namely, a ganglioside-stimulated and a ganglioside-inhibited protein kinase, phosphorylase kinase, and protein kinase C. Gangliosides also can modulate the phosphorylation of certain proteins such as myelin basic protein by substrate-directed effects. Thus, perturbation of gangliosides may confer a synchronistic action on the regulation of several protein phosphorylation systems. As a consequence, various physiological responses can be attained synergistically. Other related on-going studies are: (1) investigation of the molecular interactions involved in the formation and maintenance of myelin's multilamellar structure; (2) determination of the modes of action of neurotoxins, especially those which can interact with gangliosides; (3) elucidation of the molecular mechanisms through which gangliosides and protein phosphorylation may affect viral proliferation and infectivity; and (4) studies on the causal relationship between ganglioside-modulated protein phosphorylation systems and receptor function. Further investigation includes the isolation and characterization of the putative HIV-receptor(s) in brain and subsequent studies of their structure-function relationships.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

201 NS 02758-02 LENP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological Studies of Experimental and Human HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, DOB, laboratory, and institute affiliation)

|         |                       |                    |             |
|---------|-----------------------|--------------------|-------------|
| PI:     | H. deF. Webster, M.D. | Chief              | LENP, NINDS |
| Others: | L. Lamperth, M.D.     | Visiting Associate | LENP, NINDS |
|         | M. Martin, M.D.       | Chief              | LMM, NIAID  |
|         | J. Leonard, M.D.      | Sr. Staff Fellow   | LMM, NIAID  |
|         | D. Pezen, B.S.        | Guest Researcher   | LMM, NIAID  |
|         | B. Potts, M.D.        | Sr. Staff Fellow   | LMM, NIAID  |
|         | C. Stanislav, B.S.    | Biologist          | LENP, NINDS |

## COOPERATING UNITS (if any)

Laboratory of Molecular Microbiology, NIAID.

## LAB/BRANCH

Laboratory of Experimental Neuropathology

## SECTION

Section on Cellular Neuropathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.0

## PROFESSIONAL

1.5

## OTHER

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to supplement light and electron microscopic studies of HIV-infected experimental and human tissues with in situ nucleic acid hybridization techniques and immunocytochemical methods to investigate the distribution of HIV in the CNS and other tissues from patients with AIDS and experimental animals. Our major findings were: 1) in transgenic mice, created by J. Leonard, M. Martin (LMM, NIAID) and associates, our in situ hybridization and electron microscope studies showed that HIV-specific nucleotides were present in abnormal keratinocytes found in skin lesions. 2) Affected transgenic mice were small and had retarded CNS development, especially in neuronal populations still migrating postnatally to destinations found in mature animals (i.e., cerebellar granule cells). 3) In bone marrow cultures infected with HIV-1 by B. Potts, electron microscope and in situ hybridization studies showed that HIV-specific nucleotides were localized in cells of the promonocyte/macrophage lineage.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02759-02 LENP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Lipid Peroxidation, Protein Oxidation and Demyelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. (Name, DOE, laboratory, and institute affiliation):

|         |                         |                    |               |
|---------|-------------------------|--------------------|---------------|
| PI:     | H.S. Mickel, M.D.       | Special Expert     | LENP, NINDS   |
| Others: | C.N. Oliver, Ph.D.      | Visiting Scientist | IR, LB, NHLBI |
|         | P.E. Starke-Reed, Ph.D. | Staff Fellow       | IR, LB, NHLBI |
|         | H.deF. Webster, M.D.    | Chief              | LENP, NINDS   |
|         | R.H. Quarles, Ph.D.     | Section Chief      | LMCN, NINDS   |
|         | J. Moller, Ph.D.        | Visiting Assoc.    | LMCN, NINDS   |

## COOPERATING UNITS, if any

National Heart, Lung and Blood Institute; Laboratory of Molecular and Cellular Neurobiology, NINDS

## LABORATORY

Laboratory of Experimental Neuropathology

## SECTION

Cellular Neuropathology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL

2.0

## OTHER

.5

## CHECK APPROPRIATE BOXES:

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project seeks to determine the role of oxidative stress in demyelination and test a hypothesis that lipid peroxidation and reactive oxygen intermediates are involved in the pathogenesis of demyelinating diseases.

During FY 1989, studies included the electrolyte-induced myelinolysis model in the rat and cuprizone-induced demyelination in the mouse.

Earlier studies showed selective myelin damage occurred in lesions of the striatum, thalamus, mesencephalon, and posterior limb of the internal capsule in oxygen treated gerbils after global brain ischemia. Mongolian gerbils similarly treated demonstrated increased lipid peroxidation and increased fourteen day cumulative mortality (Mickel HS et al., 1987).







## ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Molecular Biology  
Basic Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

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## Annual Report

October 1, 1988 through September 30, 1989  
Laboratory of Molecular Biology  
Basic Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

Ernst Freese, Ph.D., Chief

The Laboratory of Molecular Biology (LMB) has continued its examination of genes encoding either enzymes that metabolize neurotransmitters or receptors for these compounds. It has also started a genetic study on the embryonic and tissue-specific control of these genes. Furthermore, the LMB has investigated the lethal effects of glutamate agonists on neurons and the dependence of this effect on the energy status and growth conditions of these cells.

### 1. Differentiation and regulation of neural gene expression.

The neurotransmitters glutamate and GABA are derived from glutamine which is mostly synthesized in astrocytes by glutamine synthetase (GS). Glutamine enters neurons where it is converted by glutaminase (GA) into glutamate and that in turn is converted by glutamate decarboxylase (GD) into GABA. After its release, glutamate is taken up by astrocytes and again converted into glutamine or broken down by glutamate dehydrogenase (GDH); GABA is degraded. The LMB has isolated cDNAs for GA and GDH and shown that they are differentially expressed in neurons and astrocytes. A three-fold induction of GA is closely correlated with the development of glutamatergic function in neurons differentiating in culture. Southern (DNA hybridization) analysis of cell hybrids containing isolated chromosomes localized the gene for GA to chromosomes 1 in mouse, 9 in rat, and 2 in humans, and the gene for GDH to chromosomes 14 in mouse and 10 in human. The expression of these genes was measured in acidotic rats in which the liver conserves bicarbonate by shifting the flow of nitrogen from urea to glutamine which in turn is transported to the kidney. Excretion of nitrogen is handled in the kidney by deamidation of glutamine (via GA) and deamination of glutamate (via GDH). Consistent with this metabolism, the LMB found that the mRNA for the two enzymes was increased in the kidney.

In situ hybridization was used to determine the distribution of the mRNA for enzymes (GS, GA, GDH, and GD) in the rat brain. Whereas the mRNA for GS and GDH was mainly located in glial cells, that for GA and GD was mostly found in neurons. In general, all areas of glutamatergic or GABAergic neurons contained the expected mRNA.

The extracellular matrix (ECM) of astrocytes stimulates neurite outgrowth from neurons in vitro. Antibody inhibition studies showed that the major neurite growth-promoting factor of the ECM is a glycoprotein called laminin. Using immunohistochemical and mRNA measurements, the LMB has shown that astrocytes produce only the B2 subunit of the three polypeptide subunits of laminin. Apparently the B2 chain suffices to stimulate neurite outgrowth. In collaboration with Dr. Yamada, the region of 800 base pairs (bp) of DNA upstream of the start site of laminin B2 transcription is used to determine the mechanism controlling the tissue-specific laminin expression. Since the production of laminin is not easily assayed quantitatively, the LMB has used for this and other control studies the following method: The genomic DNA was isolated, sequenced and the transcription start site was determined. A reporter gene for chloramphenicol transacetylase (*cat*) was attached instead of the normal reading frame (e.g., for laminin) after the enhancer/promoter region of the gene; the effect of this construct and its mutations was examined in transfected cells. In addition, footprinting of nuclear

proteins was used to identify proteins binding to specific DNA regions. For the laminin B2 gene it was found that it contains no TATA box, which is frequently found in promoter regions of genes, but does enable expression of the CAT gene in C6 glioma cells. Using a number of deletion mutants, the control region was located between -94 and +6 bp.

A similar approach has been used to study the control of glutamine synthetase (GS). The synthesis of this enzyme is induced by cortisone, insulin, cAMP, and cell-cell interaction. Full-length cDNA and genomic clones of GS in the rat were isolated and sequenced. The mRNA for GS could be detected as early as embryonic day 14, increased up to the time of birth, and later showed a second increase at the time at which type 2 astrocytes are typically made. The region upstream from the 5' start site contains a TATA box, a GC-rich region, a CCAT box, and sequences with homology to response elements to cAMP, glucocorticoid and the transcriptional activator AP2. Again using the CAT reporter gene, it was found that the region of 400 bases preceding the RNA start site apparently confers cell specificity because it allows transcription in hepatoma cells and astrocytes but not in HeLa or cervical carcinoma cells.

LMB's studies with the gene for glial fibrillary acidic protein (GFAP) brought surprising results. GFAP is found exclusively in mature brain astrocytes where it is the major subunit of intermediate-diameter filaments. The study of its control is of interest because the amount of GFAP increases greatly as a result of nerve damage. To determine the DNA sequence responsible for basal level transcription, an oligonucleotide was used to identify, by reverse transcriptase, the start site and sequence of the first 250 bases of mRNA. The results of this and numerous studies with deletions, insertions, and base alterations suggested that there are two promoter elements which are each able to initiate transcription from the same site; a combination of the two was 10 times more efficient than each one alone. One promoter is located upstream and contains a TATA box, the other novel type contains a box A-like sequence and is located downstream between +10 and +50 bp from the transcription startpoint. The TATA box usually is transcribed by RNA polymerase II and the box A area by RNA polymerase III, but in our case both promoters only used RNA polymerase II. Further studies are now underway to determine the tissue specificity of transcription.

## 2. Neurotransmitter Gene Studies

Genetic engineering methods have provided a new approach to the study of receptors for neurotransmitters. Whereas classical binding or electrophysiological studies could distinguish between different receptors only if different agonists or antagonists were found, it is now possible to isolate individual receptor genes, determine their nucleotide sequence, and thereby ascertain the evolutionary similarity and yet functional difference between different genes for a given neurotransmitter. Transient or permanent transfection of such genes into cells that do not have any of the receptors in question enables a detailed pharmacological study of a receptor encoded by a single gene. If these genes are coupled to GTP-activated G-proteins, the specificity of different proteins and their genes can also be analyzed. Furthermore, if cells large enough for electrophysiological studies are used, one can determine the ion channel properties of the isolated receptor protein.

Previous work had identified and sequenced the genes for five different muscarinic acetylcholine receptors subtypes. The LMB has continued to characterize these subtypes pharmacologically with regard to agonists and antagonists, their anatomic distribution, their selectivity of coupling to G-proteins, second messengers, and ion channels, and isolated antibodies specific for each protein subtype. Receptors m1, m3, and m5 increase small cAMP levels and phosphatidylinositol metabolism, and

open potassium channels by coupling to pertussis toxin-insensitive G-proteins. In contrast, even-numbered muscarinic receptors decrease cAMP levels by coupling to pertussis toxin-sensitive G-proteins. By producing chimeric m2-m3 receptors, the LMB has shown that a small domain of 18 amino acids suffices to reverse the coupling selectivity of m2 versus m3 receptors with respect to inositol phosphate metabolism. A major objective is to determine the genetic identity of the individual G-proteins which mediate functional responses of the muscarinic receptors. One strategy is to coexpress individual muscarinic receptors cDNAs with cDNAs for individual G-proteins. For m4 receptors, coexpression of the receptor cDNA with the G-protein Go (which is a substrate of pertussis toxin) results in receptors which are more efficiently coupled to adenylate cyclase inhibition than are m4 receptors expressed alone. Experiments are underway to determine whether the coupling between m4 and Go is direct or indirect. Using cells permanently transfected with individual muscarinic receptor genes, it is possible to identify agonists or antagonists which specifically react with the receptor proteins encoded by one particular gene or a particular group of genes. The LMB has so far identified one new agonist, the oxotremorine analog BM-5, which appears to be specific to one particular muscarinic subtype. Using *in situ* hybridization, the distribution of mRNA for the different muscarinic receptor genes has been determined. In particular, it was found that the m5 receptor gene is abundantly expressed in the dopaminergic neurons of the substantia nigra.

Based on the sequence of a dopamine D2 receptor reported recently, a series of oligonucleotide probes was used to show that the mRNA encodes both pre- and postsynaptic dopamine D2 receptors in three major ascending dopaminergic pathways: the nigrostriatal, mesolimbic, and mesocortical. Since the LMB found the mRNA only in the inner retina and not in the photoreceptors, whereas dopamine D2 receptors have been observed to be abundant in photoreceptors, the data indicate that dopamine receptors in photoreceptors may be encoded by another gene.

So far none of the three glutamate receptors (for kainate, quisqualate, and NMDA) have been isolated although they are obviously important. They are quite ubiquitous in mammalian brain; the individual glutamate analogs have been identified as neurotransmitters, and a variety of diseases has been tentatively associated with over- or underproduction of these transmitters or their receptors. The LMB has used two methods to attempt isolating the genes for these receptors. The major obstacle was the fact that most agonists or antagonists have such a low affinity for the receptor that they could not be used for receptor isolation. However, domoic acid is a kainate agonist which binds relatively strongly to the kainate receptor. Analysis of different organisms has shown that the frog brain contains a particularly high density of kainate receptor proteins. Therefore, Dr. Wenthold's group in the National Institute of Deafness and Other Communicative Diseases has used domoic acid binding as a method to isolate the kainate receptor protein from frog brain. The LMB has used the partial amino acid sequence from that protein to generate oligonucleotide probes with which a cDNA clone was isolated. This clone has been sequenced and the sequence indicates a transmembrane region expected for a receptor. The transcription and then translation of the cDNA produce a protein that binds in the expected way to the agonists or antagonists of kainate and to a highly purified kainate receptor antibody. Attempts are underway to show that this protein also has ion channel properties.

The second method of glutamate receptor gene isolation is based on both functional and pharmacological data, which predict that nicotinic acetylcholine and glutamate receptors may belong to a homologous family of genes having similar transmembrane and other properties. Therefore the LMB has used a series of oligodeoxynucleotide probes to a region of the neuronal nicotinic receptors which is expected to be in the channel lining of the receptor and screened a human retinal cDNA library



at low stringency. As one would expect, some of the clones are those for a nicotinic receptor; but 80 clones of unknown character will be analyzed for the possibility of containing the cDNA for a glutamate receptor.

Upon neuronal depolarization, depending on opening of the sodium channel, a Ca-channel opens and enables Ca ions to enter the neuron and enable the release of neurotransmitters from synaptosomes. The cDNA for one of these channel types (the L-type) has been isolated in skeletal muscle. The LMB has used this cDNA and random priming (because the mRNA is very long) to probe a cerebral cortex cDNA library to isolate portions of the Ca-channel cDNA from the brain. Hybridization studies showed that the chain is expressed in cerebral cortex, striatum, hippocampus, and retina. The gene is present in the chromosome set in only one chromosome and experiments are underway to determine the location in both human and mouse chromosomes. The LMB has also isolated a genomic library, determined the area around the start site of mRNA, and will now determine the exact location of this start site and identify promoter and enhancer regions and tissue specificity.

### 3. Neurotoxic Effects of Glutamate

When glutamate or some of its agonists are present in excess in the brain it causes neuronal death. Such conditions may be responsible for the after-effects of stroke, for Huntington's disease, or for Alzheimer's disease. The LMB has investigated the effect of glutamate on cerebral granule cells which contain NMDA receptors. In healthy brain the channel controlled by the NMDA receptor is normally blocked by magnesium in a voltage-dependent manner, i.e., magnesium prevents ion influx through the channel unless the membrane potential decreases significantly. Under physiological conditions the NMDA channel opens to permit ion flow only in response to high-frequency stimulation. When the channel is opened, both  $\text{Na}^+$  and  $\text{Ca}^{2+}$  ions flow into the cell. The magnesium block is removed when either magnesium is removed from the medium or when the cell has insufficient capability to maintain ATP, e.g., because the glucose concentration in the medium decreases below an initial value. Under those conditions, glutamate causes the NMDA channel to remain open, and the excess amounts of ions flow into the cell and kill it. Whether the cell dies due to an osmotic effect or due to an excess calcium influx which activates proteases is still under debate. The LMB also found that the presence of dexamethasone increases the sensitivity of neurons to cell death, probably because it down-regulates the glucose transporters of cells. This may explain why Sapolsky has found that elevated levels of adrenocorticosteroids render hippocampal neurons sensitive to insults and that the death of these neurons is sharply reduced in aged rats adrenalectomized at an early age.

The LMB has developed a chemically defined medium which permits the growth of granule cells in both high (25 mM) and low (5 mM) KCl. Surprisingly, cells produced NMDA receptors in high KCl but quisqualate receptors in low KCl media. It took about 4 days for the cells to change from one state to another. When the cells had developed quisqualate receptors, they were extremely sensitive to this compound, 2  $\mu\text{M}$  quisqualate being toxic. Furthermore, in high KCl, cells were ouabain-sensitive and in low KCl ouabain-resistant in what seems to be the first instance of differential expression of receptor subtypes in neurons in response to the manipulation of culture conditions.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02677-05 LMB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Differentiation and Regulation of Gene Expression in Astrocytes and Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: E. Freese, Ph.D., Chief, LMB, NINDS

Others: C. Banner, Ph.D., Senior Staff Fellow, LMB

M. Brenner, Ph.D., Expert, LMB

H. Chin, Ph.D., Senior Staff Fellow, LMB

Y. Nakatani, Ph.D., Visiting Associate, LMB

J. Mill, Ph.D., Senior Staff Fellow, LMB

J. Wujek, Ph.D., Senior Staff Fellow, LMB

F. Besnard, Ph.D., Visiting Fellow, LMB

R. King, Ph.D., Visiting Fellow, LMB

K. Mearow, Ph.D., Visiting Fellow, LMB

H. Purohit, Ph.D., Visiting Fellow, LMB

## COOPERATING UNITS (if any)

Dr. Y. Yamada, LDBA, NIDR, NIH

Dr. M. Nirenberg, LBG, NHLBI, NIH

Dr. R. Wenthold, LNO, NINDS NIH

## LAB/BRANCH

Laboratory of Molecular Biology, BNP

## SECTION

Developmental Biology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

8.3

## PROFESSIONAL:

7.6

## OTHER:

0.7

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The genes for a number of neurologically important proteins have been isolated and sequenced, and their control mechanisms are being studied. In particular, we want to understand why certain genes are expressed in neurons but not in astrocytes and vice versa. Laminin is a component of the extracellular matrix of astrocytes and promotes neurite outgrowth. In basal lamina cells laminin normally consists of 3 chains (A, B1, B2). Using chain-specific antibodies and small cDNAs, we have shown that astrocytes make only the B2 mRNA and protein of laminin. The control region responsible for this specificity is being studied. Glial fibrillary acidic protein (GFAP) is an intermediate filament protein found only in astrocytes. It is transcribed by RNA polymerase II but, in contrast to other genes, uses as promoter not only an upstream TATA box but also a downstream area containing a "box A"-like sequence. Glutamine synthetase (GS) converts in astrocytes glutamate to glutamine which then enters neurons and is there converted to glutamate or GABA which serve as neurotransmitters. The promoter for the GS gene contains a TATA box, and areas typical for control by cAMP and cortisone. Transfection of different cell lines by a 400 kb promoter area attached to a reporter gene (chloramphenicol transacetylase) showed cell specificity. The DNA area determining this specificity is being analyzed. The gene for the voltage-sensitive calcium channel (long-term opening) of the rat brain has been isolated and largely sequenced. The mRNA for this channel is surprisingly large (8.5 kb) and is present in cerebral cortex, striatum, hippocampus, and retina. Mammalian neurons have 3 different glutamate receptors (for kainate, quisqualate, and N-methyl-D-aspartate). So far none of these receptors have been isolated, the major difficulty being that the known binding compounds have a relatively low affinity. The highest affinity is that of domoate to the kainate receptor which has been used to isolate this receptor from frog brain where it is abundant. Initial partial protein sequencing of the receptor and using the equivalent oligonucleotides, a cDNA clone (KR-1) of the receptor has been isolated which contains base sequences corresponding to the amino acid sequences of the receptor. The detailed properties of this clone are being studied.

5-LMB/DIR



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02680-05 LMB

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nucleotide Sequence and Control of Glucose Dehydrogenase Operon

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: E. Freese, Ph.D., Chief, LMB, NINDS

Others: Y. Nakatani, Ph.D., Visiting Associate, LMB, NINDS

## COOPERATING UNITS (if any)

Prof. Setlow, University of Storrs, Storrs, CT

Dr. Irie, National Institute of Animal Industry, Ibaraki, Japan

## LAB/BRANCH

Laboratory of Molecular Biology, BNP

## SECTION

Developmental Biology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.3

PROFESSIONAL: 0.3

OTHER: 0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The gdh operon, which contains the gene for glucose dehydrogenase, is developmentally controlled in Bacillus subtilis. The operon is turned on only during sporulation and only in the forespore compartment. Using  $\beta$ -galactosidase as a reporter gene, we have investigated the developmental control of the gdh operon. Using deletions and site-directed mutations, we have identified the area from -10 to -35 base pairs from the start of transcription as the site which controls the onset of transcription. By in vivo and in vitro comparisons we have shown that a sporulation-specific sigma factor ( $\sigma^G$ ), which is made only inside the forespore and combines there with RNA polymerase, controls the onset of transcription. Other studies have shown that the gdh operon is not required for sporulation or germination under normal conditions. However, it is likely that germination can use alternate pathways one of which involves the operon.

\*This project has been terminated (9/89).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02365-11 LMB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Neurotransmitter-Receptor Interactions in Mammalian Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                        |   |
|---------|------------------------|---|
| P.I.:   | R.C. Henneberry, Ph.D. | Chief, Molecular Neurobiology Section, LMB, NINDS |
| Others: | P.G. Lysko, Ph.D.      | Guest Researcher, LMB, NINDS                      |
|         | A. Novelli, Ph.D.      | Visiting Associate, LMB, NINDS                    |
|         | J.A. Cox, Ph.D.        | Visiting Associate, LMB, NINDS                    |
|         | M. Voigt, Ph.D.        | NRC Fellow, LMB, NINDS                            |

## COOPERATING UNITS (if any)

Enzyme Chemistry Section, Laboratory of Neurochemistry, BNP, DIR, NINDS

## LAB/BRANCH

Laboratory of Molecular Biology, BNP

## SECTION

Molecular Neurobiology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

5.4

## PROFESSIONAL:

3.9

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have focused our studies on the mechanisms by which neurotransmitters become neurotoxins and the role of these toxins in the death of neurons characteristic of neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, lathyrism, etc. We have shown that cerebellar neurons cultured from neonatal rats express several subtypes of glutamate receptor, including the N-methyl-D-aspartate (NMDA) receptor. When this receptor is occupied by an appropriate agonist, a receptor-gated channel opens, permitting sodium and calcium influx. However, in the healthy brain this channel is normally blocked by magnesium in a voltage-dependent manner, i.e., magnesium prevents ion influx through the channel at normal membrane potential. Under physiological conditions, the NMDA channel may only permit ion flow in response to high-frequency stimulation. We have shown that the magnesium block is relieved when neurons partially depolarize in response to reduced energy levels in the neuron; decreases in adenine nucleotide levels due to glucose starvation, oxygen deprivation, or metabolic poisons cause sufficient depolarization to relieve the magnesium block of the channel. Thus, when neuronal energy levels are compromised, endogenous agonists such as glutamate can persistently open the NMDA channel resulting in excess ion influx; the increased energy demands by the pumps involved in maintaining ion gradients cannot be met in the energy-poor neurons, and neuronal death ensues via a mechanism not yet understood.

Our results provide experimental evidence for a mechanism which may trigger the transition of endogenous glutamate from neurotransmitter to neurotoxin; this mechanism may have important implications for a variety of neurodegenerative disorders.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02755-02 LMB

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Amino Acid Neurotransmitter Metabolism

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: C. Banner, Ph.D. Senior Staff Fellow, LMB, NINDS

Other: A. Novelli, Ph.D. Visiting Associate, LMB, NINDS

## COOPERATING UNITS (if any)

Dr. B. Mock, Lab. of Genetics, NCI; Dr. M. Schneider, LNO, NINDS; Dr. N. Curthoys, Univ. of Pittsburgh Sch. of Med.; Dr. A. Bale, CEB, NCI; Dr. J. Thomas, Searle/Monsanto, St. Louis, MO; Dr. W. McBride, CEB, NCI

## LAB/BRANCH

Laboratory of Molecular Biology, BNP

## SECTION

Developmental Biology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glutamate is an excitatory neurotransmitter in the mammalian central nervous system and the precursor of the inhibitory neurotransmitter GABA. The enzymes which control glutamate's metabolism must have special roles in the brain different from their roles in other organs. Neuronal glutamate is largely derived from glutamine by a glutaminase (GA) which is similar or identical to that found in kidney, but different from liver glutaminase. Glutamate dehydrogenase (GDH), a key enzyme for ureagenesis and pH homeostasis in liver and kidney, is abundant in brain but without a known role. We have isolated cDNAs for GDH and GA, and shown that they are differentially expressed in neurons and astrocytes; we have also shown that a three-fold induction of GA is closely correlated with the development of glutamatergic function in neurons differentiating in culture. We have also found that GA and GDH mRNAs are induced 6-fold and 3-fold, respectively, in the kidneys of acidotic animals. We have mapped GDH genes (GLUD) to chromosomes 14 in the mouse and 10 in humans, and GA genes (GLS) to chromosomes 1 in mouse, 9 in rat, and 2 in humans. We have identified two additional GLUD loci in humans which may be pseudogenes. The brain from a patient diagnosed as "GDH-deficient OPCA" was shown to have normal levels of GDH enzyme and mRNA, casting doubt on the association of GDH deficiency with neurodegenerative disease.

\*This project has been terminated (9/89).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02800-01 LMB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotransmitter Gene Studies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                    |                     |            |
|---------|--------------------|---------------------|------------|
| P.I.:   | M.R. Brann, Ph.D.  | Senior Staff Fellow | LMB, NINDS |
| Others: | T. Stormann, Ph.D. | IRTA Fellow         | LMB, NINDS |
|         | T.J. Murphy, Ph.D. | IRTA Fellow         | LMB, NINDS |
|         | D. Weiner          | HHMI Fellow         | LMB, NINDS |
|         | J. Wess, Ph.D.     | Special Volunteer   | LMB, NINDS |
|         | B. Novotny, M.S.   | Physiologist        | LMB, NINDS |

## COOPERATING UNITS (if any)

S. Jones, J.L. Barker, LNP, NINDS; W. Simonds, A. Spiegel, MPB, NIDDK; H. Arnheiter, LMG, NINDS; T. Bonner, LCB, NIMH; A. Levey, JHU; J. Ellis, Univ. VT; S. Jones, D. Gdula, N. Nash, Rec. Genet., CRADA

## LAB/BRANCH

Laboratory of Molecular Biology, BNP

## SECTION

Developmental Biology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

4.7

## PROFESSIONAL:

2.7

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our work involves the study of genes encoding receptors which bind the neurotransmitters acetylcholine, dopamine, and glutamate. Previous work led to the molecular cloning, sequencing and expression of a family of muscarinic acetylcholine receptor subtype genes. We have continued to characterize these subtypes pharmacologically with regard to agonists and antagonists, anatomic distribution, their selectivity of coupling to G-proteins, second messengers and ion channels, and the development of antibodies specific for each protein subtype. Based on the recently reported sequence of a rat cDNA which encodes a dopamine receptor, we have mapped the distribution of the corresponding mRNA in rat retina and brain, and have cloned, sequenced and expressed a cDNA which encodes the human homolog of this receptor. This work has provided evidence that dopamine receptors, like muscarinic receptors, may be derived from a family of genes. We are attempting to clone the other members of the family, and to study the selectivity of coupling of the cloned dopamine receptors to G-proteins, second messengers, and ion channels. Finally, based on recently reported sequences of the rat cDNAs which encode neuronal nicotinic acetylcholine receptors, we have cloned some of the human homologs of these receptors. Based on a hypothetical evolutionary similarity between glutamate and nicotinic acetylcholine receptors, we are attempting to clone the glutamate receptors by homology cloning.



**TAB 6 -- LABORATORY OF VIRAL AND MOLECULAR PATHOGENESIS (LVMP)**





October 1, 1988 through September 30, 1989

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989  
Laboratory of Viral and Molecular Pathogenesis  
National Institute of Neurological Disorders and Stroke

Monique Dubois-Dalcq, M. D., Acting Chief

The Laboratory of Viral and Molecular Pathogenesis (LVMP) was created last October (1988) to regroup members of three sections of the former Laboratory of Molecular Genetics (LMG) with those of one section of the former Infectious Diseases Branch (IDB).

The new laboratory investigates the viral and molecular bases of nervous system diseases using the most advanced techniques of molecular and cell biology. The laboratory studies the cell and molecular biology of neural cells in vitro and in vivo, characterizes mutations in the myelin gene and studies the cells involved in myelin repair in mice and men. An important goal of LVMP is to elucidate mechanisms of nerve cell dysfunction in human diseases caused by RNA and DNA viruses, especially human retroviruses (HIV, HTLV-1) and other viruses associated with them, such as JC papova virus and herpes virus. The mechanisms of demyelination in acquired immunodeficiency syndrome, tropical spastic paraparesis, multiple sclerosis, and progressive multifocal leukoencephalopathy are being studied. The laboratory attempts to elucidate how human neurotropic viruses get to the brain and which viral and host factors determine the final outcome of infection. Long-term goals are to find ways to stop virus entry in nerve cells, to inhibit viral cytopathogenicity and persistence, and enhance nervous system repair. The new laboratory gathers scientists with expertise in the cellular and molecular biology of glial cells and others with expertise in the molecular biology and pathogenesis of neurotropic viruses. It has maintained the breadth of expertise in cellular and molecular biology which was characteristic of the LMG and there is an ongoing discussion of the cellular and molecular aspects of biological phenomena between the different members of the laboratory. In addition, the laboratory has an active transgenic mice facility which has started several collaborations with other NINDS and NIMH laboratories.

A main research effort on AIDS virus entry and neurotropism has been started in LVMP this year. This has required the creation within the 5C corridor (Bldg. 36) of an AIDS facility (5C10) where safety rules are strictly applied. We have developed new techniques for making chimeric molecules and for growing human nerve cells from adult brain (see below); we have established numerous contacts with AIDS research laboratories within and outside NIH to gather tools and reagents, and have elaborated new research strategies to tackle the mechanisms by which human retroviruses affect the nervous system. An IDB project on murine AIDS directed by Dr. Klinman was continued in LVMP this year but ended with the departure of this senior investigator in July 1989.

While this retrovirus program was started, neurobiologists, and molecular biologists in the laboratory, pursued their research on: the characterization of the progenitor cells of oligodendrocytes and the factors that promote their migration, growth and differentiation; on an animal model for demyelination followed by remyelination; the characterization of the mutations in the proteolipid protein (PLP) gene that cause dysmyelination; the characterization and mode of action of the transcription factor HOX 1-3; the role of the Mx protein in the protection against influenza infections both in vitro and in vivo; the molecular biology of JC virus which causes progressive multifocal leucoencephalopathy (PML, a disease often associated with AIDS); and the transcription factors conferring glial cell specific expression of JC virus and PLP. I will first briefly describe the highlights of the research projects listed above and then summarize those of the new retrovirus research projects.

Several milestones have been accomplished this year in our studies of glial cell biology in development and regeneration. First, we were able to study gene expression at the molecular level on rat purified O-2 progenitor and this has allowed to analyze how two growth factors, PDGF and FGF, can regulate gene expression in these cells and determine their fate. O-2A lineage cells were also isolated and cultured from adult human brain allowing future analysis of the O-2A lineage in man. Moreover, the characterization of the cells responsible for remyelination in rodents has progressed considerably with the identification of dividing O-2A progenitor in remyelinating mice and the successful culture and characterization of O-2A lineage cells in vitro from these animals. Together with the observation that specific myelin gene transcripts are reexpressed coordinately in remyelination, these studies point to an important role in myelin regeneration of the O-2A progenitor cells persisting in the adult CNS.

In the studies of dysmyelination in man and animals, Dr. Hudson and colleagues were the first to identify the defect in two distinct Pelizaeus-Merzbacher families where a different point mutation was discovered in each pedigree. Similarly distinct missense mutation in *Jimpy<sup>msd</sup>* mice and the shaking pup were identified. The model for the structure of PLP in the oligodendrocyte plasma membrane indicate that the alpha-helical domains of the protein which seem critical in mediating homophilic interactions and myelin compaction are affected by several of these mutations. Progress has also been made in the characterization of the regulatory signals that control PLP gene expression since a 4 kb fragment of upstream sequence could confer high levels of specific PLP expression in the NS of one line of transgenic mice. In addition, several sequences were identified in this promoter and shown to be binding sites for brain nuclear proteins in footprinting experiments. A 1 kb sequence upstream of the initiation site appears to contain all the cis-regulatory signals necessary for efficient transcription in a glial cell line. Experiments are in progress to clone the genes encoding specific regulatory proteins binding to these regions.

Important progress has been made this year on the characterization and expression of the HOX 1.3 mammalian homeobox gene by Dr. Odenwald, and colleagues. This protein comes in a variety of phosphorylated forms which all bind to a specific target sequence found in many eukaryotic cellular and viral promoters (and also in origins of DNA replication). A critical step in the identification of the genes controlled by HOX 1.3 was the establishment of a stable

mouse L cell line in which HOX 1.3 cDNA has been placed behind the interferon-inducible Mx promoter. Differential libraries from induced and uninduced L cells are now being prepared and screened. In addition, the Mx promoter-Hox1.3 construct will be placed into the germ line of transgenic mice. One should be able to induce expression of HOX 1.3 in these mice and study its effect at different times during development.

In a remarkable accomplishment of the transgenic mice technology, Dr. Arnheiter and colleagues have been able to make a line of animals resistant to a viral infection. More specifically, the Mx gene, with its interferon-inducible promoter, conferred "inducible" expression of Mx protein in virtually all organs of transgenic mice and this resulted in resistance of these mice to influenza virus infection. Importantly, these studies show that a single intracellular host factor, the Mx protein, is sufficient to protect mice against an otherwise lethal infection, a phenomenon that can be called intracellular immunity. [The influenza A virus strain used in these studies is the neurotropic Wilson-Smith strain or NWS strain]. The Mx genes are expressed from yeast to man and may vary in function depending on their nuclear or cytoplasmic location. This question is presently studied in rat cells (Meier) where chimeric molecules will be tested in order to determine the active site of the Mx molecule. Thus the Mx system allows one to delineate host genetic factors controlling viral pathogenesis.

In their molecular studies of the neurotropic papova virus JC, Dr. Major and colleagues have successfully identified a nuclear factor -1 (NF-1) like protein which preferentially binds to three regions in the JCV promoter enhancer promoter and does not bind to SV40. They also demonstrated that JC virus can replicate productively in astrocytes as well as oligodendrocytes. Moreover, by altering the JC regulatory sequences (making a chimeric SV40-JCV promoter/enhancer region), the host range of the virus was extended from humans to monkeys. When recombinant JC virus is injected into immunocompromised monkeys it causes lesions in the deep white matter, a syndrome very similar to that seen in PML. Ongoing studies of this group take advantage of their long standing expertise in culture and characterization of human fetal cells to analyze HIV entry and replication in these cells.

### Projects on Human Neurotropic Retroviruses

There are at least two major viral pathogens for the nervous system among human retroviruses: human immunodeficiency virus (HIV) which can cause various neurological syndromes including dementia in AIDS patients, and human T cell leukemia virus (HTLV-1) which is associated with tropical spastic paraparesis (TSP). We have started a series of projects on HIV entry, replication and assembly in nerve cells in vitro, as well as on the effects of retroviral genes on the function of cells of the nervous and immune system in vivo in transgenic mice. In addition a new strategy for making pseudotypes that may kill HIV infected cells has been designed. Dr. Schubert, et al., have indeed set out to study the interaction of the HIV envelope gp 160 with the HIV receptor protein CD4 using chimeric molecules and pseudotypes in a newly designed strategy. Using a rapid and precise gene fusion method, they constructed a recombinant chimeric molecule consisting of the CD4 ectodomain fused to the



transmembrane and cytoplasmic domain of the vesicular stomatitis (VSV) glycoprotein G. This molecule, when expressed in Hela cells was found to be functional, i. e., it was able to produce fusion with gp160 expressing Hela cells. Moreover this chimeric HIV receptor was used to generate a novel pseudotype virus which contains the VSV genome and the chimeric molecule in its envelope. Such virus appears to be able to infect specifically an HIV infected T cell line, presumably through the binding of the CD4 ectodomain to gp160 expressed on the HIV infected cells. Once this new pseudotype is further characterized and the specificity of its tropism for HIV infected cells confirmed, this approach could be used to place other viral genomes inside envelopes with chimeric molecules that can specifically bind to HIV infected cells. Such pseudotypes might interfere with HIV replication and therefore control its expression and spreading.

An essential question inherent to the spreading of HIV to the nervous system is the identification of the receptor molecule present on nerve cells. CD4 is expressed in the brain, but some of the nerve cell types may not express a full transcript coding for the CD4 ectodomain binding site for HIV. We have set out to explore this question using a variety of molecular techniques and nerve cell purifications (Dubois-Dalcq, et al). In collaboration with the Neurosurgery Branch in our Institute (Dr. Kufta) we have developed a reliable in vitro system to culture human microglial cells, astrocytes, and oligodendrocytes. Using various HIVs strains (including one HIV2), we found so far that only macrophage tropic strains actively replicate in our cultures and that the major nerve cell type infected are the microglial cells. In fact the giant cells typically seen in the brain of AIDS patients with subacute encephalitis can form by fusion of infected microglial cells in our dishes. This suggests that, after AIDS virus infected macrophages have reached the brain, virus could readily spread to the resident microglial cells which are scattered throughout CNS. Future experiments are aimed to determine whether astrocytes and oligodendrocytes may be latently infected (as was described for glioma cells). We will also study this year the nerve cell tropism of HTLV-1 in this in vitro system. In HTLV-1 infection, it is not understood why the virus causes T cell leukemia in some patients and TSP in others. One possibility is that a single viral regulatory protein, e.g. ,TAX, can cause cell dysfunction when expressed in lymphocytes or in nerve cells. To address this question, Dr. Arnheiter and colleagues have introduced into the mouse germ line TAX under the control of two different promoters: the IL2 receptor promoter (which is known to be positively regulated by TAX) and the interferon inducible Mx promoter (see above). Ongoing analyses have revealed expression of the Mx TAX construct but no phenotypic changes have yet developed in these mice. The question whether regulatory proteins of HIV and HTLV-1 can cause nerve cell dysfunction in vivo will be addressed this year by using nerve cell specific promoter directing expression of these genes in the nervous system of transgenic mice.

These programs on retroviruses neurotropism are producing promising results, yet they need expansion with the hiring of highly experienced molecular retrovirologist(s). This will

be essential to promote our understanding of the molecular interactions between retroviruses and nerve cells and to enhance the training in retrovirology of those scientists who have decided to shift their research interests. It is also essential that the laboratory has functional and adequate space to perform safely the work with these human retroviruses.

Dr. Schubert has filed for a patent for his invention "Rapid and Precise Sequence Specific Fusion of DNAS" (5/5/89, E 191-89).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02034-17 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Myelin-Forming Cells *In vitro* and *In vivo* Including Remyelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.

M. Dubois-Dalcq, M.D. Act. Chief, LVMP LVMP, NINDS

Others:

R. McKinnon, Ph.D. Guest Worker LVMP, NINDS H. Dorn, B.S. Biologist LVMP, NINDS.

C. Jordan, Ph.D. Sr. Staff Fellow LVMP, NINDS R. Rusten Biol Lab. Techn. LVMP, NINDS

R. Armstrong, Ph.D. IRTA LVMP, NINDS

B. Watkins, Ph.D. Visiting Fellow LVMP, NINDS

## COOPERATING UNITS (if any)

K.V. Holmes, Dept. Pathology, USUHS; Drs. T. Matsui and S. Aaronson, LMG, NCI; and Dr. K. Kuffa, Neurosurgery Branch, NINDS.

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Section on Neural and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

4

## PROFESSIONAL

2.7

## OTHER

1.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The myelin sheath is essential for normal conduction in major nerve tracks. Therefore neurological dysfunction occurs in demyelinating diseases such as Multiple Sclerosis (MS) and viral encephalitis. We study how myelin forming cells can develop and regenerate both in vitro and in vivo. We analyze at the molecular level how PDGF and FGF regulate the growth and differentiation of the oligodendrocyte-type 2 astrocyte (O-2A) progenitor cells. FGF, which is a more potent mitogen for these cells than PDGF, produces an increase in PDGF receptor expression and an inhibition of differentiation in the rat. We have successfully cultured O-2A lineage cells from adult human brain and are studying the cells and factors necessary for their regeneration in vitro. Since cell migration is an important component of glial cell development and regeneration, we measure glial cell migration (in vitro), in a 2 chambers assay. Astrocytes migrate in a direct and dose dependent manner towards laminin and C5A. We are presently examining which molecules can trigger the O-2A progenitor cells migration. Orchestration of these progenitor migration appears essential to developmental myelination.

Our studies of a demyelinating disease caused by a corona virus (A59) in C57 Bl mice have revealed that the widespread distribution of the virus seen in the first weeks faced to near complete clearing at 3 weeks post inoculation (PI) at the peak time of demyelination. Simultaneously, a progressive increase in myelin gene transcripts occurs in a synchronized manner and with a cellular distribution reminiscent of developmental myelination. In the infected spinal cord, O-2A progenitors start to proliferate at 1 week PI and appear to later generate both oligodendrocytes, and type 2 astrocytes instrumental in remyelination. One can isolate large numbers of O-2A lineage cells from these remyelinating animals (compared to control) and characterize them in vitro. A substantial proportion of these cells spontaneously divide but do not grow faster in the presence of PDGF. FGF inhibits oligodendrocyte differentiation while IGF1 favors that event. Thus oligodendrocyte regeneration is happening in this demyelinating model. Further studies are planned to characterize the factors responsible for precursor proliferation and differentiation in vivo. Such studies may lead to the design of ways to enhance myelin regeneration in human demyelinating diseases.

\*Formerly in LMG

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02528-08 LVMP \*

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Regulation of Myelin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. L. D. Hudson, Ph.D.

Senior Staff Fellow

LVMP, NINDS

Others: S. Gencic, Ph.D.

Guest Worker

LVMP, NINDS

N. Nadon, Ph.D.

Staff Fellow

J. Kim, Ph.D.

NRC Fellow

LVMP, NINDS

J. Berndt, B.S.

Microbiologist

LVMP, NINDS

## COOPERATING UNITS (if any)

Dr. Heinz Arnheiter, Section of Viral Pathogenesis, LVMP.

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

3.58

## PROFESSIONAL

2.58

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to define the regulatory signals that control myelination, the event where oligodendrocytes and Schwann cells extend processes that enwrap and ensheath axons. We have identified several sequences in the promoter region of the myelin proteolipid protein (PLP) gene, some of which are conserved among the myelin-specific genes, that act as binding sites for nuclear proteins isolated from brain. The contention is that these putative regulatory proteins make with the cis elements have been precisely defined by footprinting techniques. In addition, the function of these cis elements has been assayed in cultured glial cells using plasmid constructions containing the various cis elements next to a reporter gene. Constructions have also been introduced into the germ line of mice to assay tissue specificity and developmental regulation in an in vivo system. A 4 kb fragment of upstream sequence was sufficient to confer maximal levels of PLP expression in a tissue-specific manner in one line of transgenic mice, providing a baseline for subsequent deletions and mutations of the promoter region.

PLP is an extremely conserved protein, as might be expected for a protein that provides the underpinning of the myelin sheath, and point mutations creating amino acid substitutions in this protein are present in a number of X-linked dysmyelinating disorders. We were the first to identify the defect in a human dysmyelinating disease, sequencing the PLP gene in two distinct Pelizaeus-Merzbacher families to discover a different point mutation in each pedigree. The mutations support the model we developed for the structure of PLP in the oligodendrocyte plasma membrane, as several of the substitutions are located in the alpha-helical domains of the protein proposed to be critical in mediating homophilic interactions that may contribute to the architecture of the myelin sheath.

\*Formerly in LMG



## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02698-04 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Mammalian Homeodomain Proteins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.

W. Oldenwald

Staff Fellow

LVMP, NINDS

Others:

J. Garbern

Medical Staff Fellow

LVMP, NINDS

W. F. Greene

HHMI-NIH Res. Fellow

LVMP, NINDS

H. Arnheiter

Visiting Scientist

LVMP, NINDS

S. Smith

Summer Student

LVMP, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Viral Pathogenesis Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.4

## PROFESSIONAL:

3.2

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Many of the proteins whose dysfunction disturbs embryogenesis of the fly share a characteristic stretch of 60 amino acids called the homeodomain. Mammals likewise express homeodomain containing proteins. Based on their nuclear localization, their capacity to bind to specific DNA sequences, and their relatedness to some well-characterized transcription factors, it is suggested that homeodomain proteins are involved in regulating gene expression in mammalian cells.

In our effort to determine the biofunction of mammalian homeodomain proteins, we have cloned, sequenced and expressed the genes for two murine proteins, Hox 1.3 and Hox 1.2. The Hox 1.3 protein comes in a variety of phosphorylated forms all of which bind to a specific target sequence found in many eukaryotic cellular and viral promoters, and also in origins of DNA replication. The Hox 1.2 protein likewise comes in a variety of immunoreactive forms, but the nature of this variability has not yet been characterized. To identify target genes regulated by Hox 1.3, we have placed a Hox 1.3 cDNA behind the interferon-inducible Mx promoter, and we have produced stable mouse L cells in which the baseline expression of Hox 1.3 is negligible and which can be induced to produce high levels of this protein by a 3-6 hour exposure to mouse interferon alpha/beta. We have then gone on to prepare cDNA libraries from induced control cells (not expressing Hox 1.3) and both induced and non-induced Hox 1.3 cell lines. These libraries are now being screened for cDNAs representing mRNAs enriched in presence of Hox 1.3. These experiments are ultimately aimed at understanding the network of gene regulation that is responsible for the generation and maintenance of a functioning organism.

\*Formerly in LMG

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02742-03 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Viral Pathogenesis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. H. Arnheiter, M.D.

Visiting Scientist

LVMP, NINDS

Others: E. Meier, Ph.D.

Staff Fellow

LVMP, NINDS

Susan Skuntz

Biol. Lab. Tech.

LVMP, NINDS

## COOPERATING UNITS (if any)

Professor Charles Weissmann, Institute for Molecular Biology I, University of Zurich, Switzerland.

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Viral Pathogenesis Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

1.5

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Mx transgenic mice: The Mx1 protein of mice is a 72 kd nuclear protein that is known to protect cultured cells specifically against infection with influenza viruses. To test whether this protein protects mice against influenza virus in vivo, we introduced an Mx1 cDNA into the germ line of Mx protein negative, influenza virus susceptible mice, and analysed the resulting transgenic mouse lines for Mx1 protein expression and susceptibility to infection with a neurotropic influenza virus. Placing the Mx cDNA under the control of the constitutive SV40 promoter resulted in transgenic mice which expressed little if any Mx1 protein and remained virus susceptible. Placing the cDNA under control of the virus/interferon inducible Mx promoter (the promoter that controls endogenous Mx1 expression in mice naturally positive for Mx) gave transgenics which expressed Mx1 protein in virtually all organs, provided they were induced with either interferon or virus. These animals were resistant to influenza infection. These studies show that a single host factor, Mx, is sufficient to make mice immune against an otherwise lethal infection. This phenomenon can be addressed as intracellular immunity as opposed to acquired, antibody mediated or cellular immunity.

2. The biological significance of the Mx system in other animals and humans: The Mx system is a gene family with members found from yeast to man. In rats, e.g., we have identified three Mx related genes: one codes for a nuclear protein which protects, and two for cytoplasmic proteins which do not protect against influenza virus. Humans also have at least two Mx genes. Both code for cytoplasmic proteins, none of which has a high level activity against influenza virus.

The analysis of the Mx system in vertebrates allows us to delineate host genetic factors controlling viral pathogenesis and to design means by which to interfere with viral spread. In addition, these interferon regulated proteins may have other physiological functions, the elucidation of which may help us understand the interferon system.

\*Formerly in LMG



## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02789-01 LVMP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Neurotropism of Human Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.

M. Dubois-Dalcq, M.D. Act. Chief, LVMP LVMP, NINDS

Others:

B. Watkins, Ph.D. Visiting Fellow LVMP, NINDS

C. Jordan, Ph.D. Sr. Staff Fellow LVMP, NINDS

H. Dorn, B.S. Biologist LVMP, NINDS

W. Kelly Chemist LVMP, NINDS

R. Rusten Biol. Lab. Tech. LVMP, NINDS

## COOPERATING UNITS (if any)

Dr. K. Kuffa, Neurosurgery Branch, NINDS. Dr. B. Potts, Molecular Virology Lab., NIAID. (now at Repligen, Boston, MA).

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Section on Neural and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS.

3.5

## PROFESSIONAL

2.3

## OTHER

1.2

## CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

AIDS patients frequently show neurological symptoms (at various phases of the disease) such as meningitis, neuropathies, myelopathy and dementia. The latter appears to coincide with a subacute encephalitis with demyelination, gliosis, and giant cells containing viral mRNAs of HIV (human immunodeficiency virus), the etiological agent of AIDS.

Our approach to study AIDS virus neurotropism has been to develop a reliable in vitro system of differentiated nerve cells derived from adult brain. Such cultures contain microglial cells, the resident macrophages of the brain, Type 1 and 2 astrocytes as well as oligodendrocytes. We have first tested the ability of T cell and macrophage HIV isolates to replicate in these cultures. So far only the macrophage tropic strains were found to cause productive infection, with the virus progressively fusing and killing the microglial cells over a period of several weeks. No evidence of productive infection of other neighboring glial cells in the culture have been found so far. These results suggest that microglia are primarily responsible for the formation of multinucleated syncytia seen in the brains of AIDS patients. We are currently investigating whether astrocytes are latently infected as previously described for glioma cells.

An important question also under study is whether the CD4 molecule, the receptor for HIV on T4 cells, is involved in virus attachment and entry into nerve cells. If it isn't, it would be crucial to determine which other receptor molecule is involved in nerve cell infection by HIV. Finally, we will also study the infection of human nerve cells with HTLV-1, which causes tropical spastic paraparesis, to determine if it can productively infect any of the nerve cell types described above.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02790-01 LVMP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Viral Proteins in Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. H. Arnheiter, M.D.

Visiting Scientist

LVMP, NINDS

Other: E. Tournier-Lasserre, M.D.

Visiting Fellow

LVMP, NINDS

## COOPERATING UNITS (if any)

Dr. Flossie Wong-Staal, Laboratory of Tumor Cell Biology, National Cancer Institute.

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Viral Pathogenesis Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS.

1.3

## PROFESSIONAL:

1.3

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HTLV-1 is a human retrovirus that is associated with either a form of adult T cell leukemia or TSP, a demyelinating disease of the central nervous system. Its genome codes for a 41 kd nuclear protein, TAX, whose expression is required to activate the viral LTR promoter. Besides its action on the viral genome, TAX is known to stimulate in cultured cells the transcription of IL-2, IL-2 receptor, GM-CSF and c-fos mRNAs. To test what effect the TAX protein might have on a host organism in vivo, we placed a TAX cDNA under control of the well studied human IL-2 receptor promoter, and another TAX cDNA (which includes the overlapping coding region for the rex protein) under control of the ubiquitous, dsRNA inducible Mx promoter, and introduced these constructs into the germ line of mice. Eleven IL-2 receptor-TAX and seven Mx-TAX transgenic founders were obtained. To date, all founders (aged 2-6 months) are healthy. Transgenic offsprings from three Mx-TAX founders expressed low to moderate levels of TAX mRNA after induction with 50 micrograms of poly IC. Offspring of two IL-2 receptor-TAX founders so far tested did not express transgene mRNA in either normal or PHA stimulated cultured spleen cells. Further analysis is needed to determine the suitability of the human IL-2 receptor promoter in transgenic mice.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 01983-18 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*\*

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Human Neurotropic Virus Infections, JCV and HIV-1\*\*\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.

E. O. Major, Ph.D. Section Chief LVMP, NINDS

Others:

K. Amemiya, Ph.D. Special Expert LVMP, NINDS S. A. Houff, M.D. Clin. Assoc. LVMP, NINDS

J. Assouline, Ph.D. Staff Fellow LVMP, NINDS R. Traub, B.A. Microbiologist LVMP, NINDS

B. Curfman, B.S. Microbiologist LVMP, NINDS D. Vacante, Ph.D. Staff Fellow LVMP, NINDS

L. Durham, M.S. Biologist LVMP, NINDS

G. Elder, M.D. Senior Staff Fellow LVMP, NINDS

## COOPERATING UNITS (if any)

Microbiological Associates, Inc., Rockville, MD

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Section on Molecular Virology and Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

9.2

## PROFESSIONAL:

6.0

## OTHER:

3.2

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating disease caused by the infection of the myelin producing oligodendrocytes by JC virus. We have shown that in addition to these cells, human fetal astrocytes (HFA) can support the growth of JCV in culture. The infection of these cells was determined by measuring early viral gene expression, JCV DNA replication, and an increase of viral particles. To study the restricted host range exhibited by JCV a chimeric polyomavirus genome was constructed between the sequences of the simian virus 40 (SV40) inserted on the late side of the JCV promoter/enhancer region. An extensive deletion had occurred in the newly constructed chimeric genome. This deleted chimeric polyomavirus showed an extended species and cell-type host range. Besides replicating in embryonic kidney cells, infectious virus was produced from fetal and adult rhesus monkey glial cells. Since the JCV T protein was left intact in this chimeric virus, extension of the JCV host range is most likely attributable to the changes in the regulatory region. We also initiated a study to identify host cellular factors which may be involved in the regulation of expression of the JCV genome. We demonstrated by gel retardation assays and competitive binding studies that a specific host factor was able to preferentially bind to the JCV promoter/enhancer region and not that of SV40. This host factor has been identified as a nuclear factor-1 (NF-1) like protein factor which is a multifunctional protein involved in both initiation of DNA replication and transcription. We have identified three sites in the JCV regulatory region which contain NF-1 binding sites. NF-1 may play a role in the restricting of the host range of JCV at the molecular level.

\*Formerly in ID

\*\* Transferred from ID on 10/88

\*\*\* Formerly: "Chronic Viral Infections - Molecular Biology of Human JC Virus"

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02026-17 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Viral Nucleic Acid Synthesis in Animal Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P. I. M. Schubert, Ph.D.

Section Chief

LVMP, NINDS

## Others:

G. Harmison II, B.S.

Chemist

LVMP, NINDS

D. Blondel, Ph.D.

Visiting Fellow

LVMP, NINDS

B. Joshi, Ph.D.

Senior Staff Fellow

LVMP, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Viral Replication Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

## CHECK APPROPRIATE BOX(ES):

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated. (10/88)

\*Formerly in LMG



## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02791-01 LVMP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Replication and Pathogenesis of Enveloped Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. M. Schubert, Ph.D.

Section Chief

LVMP, NINDS

## Others:

G. Harmison II, B.S.

Chemist

LVMP, NINDS

D. Blondel, Ph.D.

Visiting Fellow

LVMP, NINDS

B. Joshi, Ph.D.

Senior Staff Fellow

LVMP, NINDS

## COOPERATING UNITS (if any)

Dr. Christopher C. Widnell, University of Pittsburgh School of Medicine, Pittsburgh, PA., Dr. Yong Kang, University of Ottawa, Ottawa, Canada.

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION

Viral Replication Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.63

## PROFESSIONAL:

2.58

## OTHER:

1.05

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrecouped type. Do not exceed the space provided.)

The emphasis of our research has shifted much beyond the scope of the original project, from negative strand viruses to other enveloped RNA viruses and in particular the AIDS virus (HIV):

1. A rapid and precise gene fusion method was invented. We have filed for a U.S. patent. This gene fusion method was used to construct and express a recombinant chimeric protein consisting of the ectodomain of the human CD4 protein (HIV receptor) precisely fused to the transmembrane and cytoplasmic domains of the vesicular stomatitis virus (VSV) glycoprotein G. A vaccinia virus recombinant was generated which expresses this CD4/G fusion protein. Coexpression of the chimeric protein and the HIV envelope protein leads to the formation of giant multinucleated cells, demonstrating functionality as an HIV env receptor.
3. Conditions were established which allow efficient insertion of this chimeric HIV receptor into the envelope of VSV, thus generating a pseudotype virus.
4. A novel pseudotype virus was generated with this system consisting of VSV which carries a chimeric CD4/G HIV receptor protein on its surface. Most importantly, this pseudotype virus specifically infects HIV infected but not uninfected cells. This is the first demonstration that an exchange of receptor and viral glycoprotein between the host cell and the viral membrane is possible and that this exchange allows efficient membrane fusion leading to infection. In the future we plan to base much of our studies on this important finding. We will generate equivalent recombinant defective HIV and SIV pseudotype viruses. This approach will be evaluated with respect to gene therapy for AIDS as well as hereditary diseases.
5. Efficient pseudotype formation is dependent on the specific interaction between the cytoplasmic domains of the viral glycoprotein with its matrix protein, M for VSV; VPU for HIV? We found that the expression of the matrix protein of VSV causes a disorganization of the cytoskeleton primarily affecting microtubules. Surprisingly, we found that the viral polymerase protein L disorganizes actin. We suspect that the disorganization of the cytoskeleton may be an important mechanism for efficient transport of nucleocapsids during the budding of enveloped RNA viruses.
6. Preliminary studies suggest the viral NS phosphoprotein of VSV is the key protein for the mechanism of heterotypic autointerference caused by defective interfering particles.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02756-02 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989 \*\*

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Neurologic and Systemic Manifestations of Retrovirus and Autoimmune Mediated Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P. I.: D. M. Klinman, M.D., Ph.D.

Senior Staff Fellow

LVMP, NINDS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

## SECTION-

Viral Pathogenesis Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

1

## PROFESSIONAL

1

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Our research is broadly divided into studies concerning 1) the role of autoantibodies in the neurologic manifestations of retrovirus-induced diseases, and 2) regulatory abnormalities of the immune system which lead to the production of pathogenic autoantibodies.

1. Retrovirus-induced neurologic disease. We have developed ELISA and ELISA-spot assays to detect the production of autoantibodies reactive with cells of neuronal, glial and astrocyte origin. Using these new assays, we have identified elevated levels of anti-neuronal antibodies in AIDS patients with neurologic manifestations of HIV infection. Ongoing studies suggest that the concentration of these antibodies, titers of HIV antigen, levels of anti-HIV antibodies, and clinical manifestations of disease are all reduced following treatment with AZT.

Mice inoculated with LP-BM5 type C retroviruses develop a syndrome with marked similarities to human AIDS (known as murine AIDS or MAIDS). We have shown that the humoral manifestations of LP-BM5 infection occur in three non-discrete stages, marked initially by polyclonal B cell activation, then by hypergamma-globulinemia, and finally by generalized immunosuppression. We are now analyzing the influence of specific T cell derived lymphokines on the activation of these B cells.

2. Systemic abnormalities in B cell activation. Using murine models of SLE, we are investigating the regulatory abnormalities which lead to the production of autoantibodies. We have found that B cell hyperactivation precedes the onset of clinically detectable disease. We have shown that this hyperactivation process is polyclonal in nature and appears to be lymphokine dependent. We are finding that changes in autoantibody affinity and isotype occur late in the disease process, and may correlate with autoantibody pathogenicity.

\*Formerly in IDB.

\*\*Project will terminate 9/89.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02714-04LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*\*

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Degenerative Process of the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.A. Lazzarini, Ph.D. Former Chief, LMG, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Viral and Molecular Pathogenesis

## SECTION

Neural and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

\* Project was transferred to the LVMP 10/88 from LMG

\*\* Project was terminated on 10/1/88 due to the departure of the P.I.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS-02532-07 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*\*

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Study of AIDS and SAIDS: Neurological Findings and Etiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John L. Sever, M.D., Ph.D. Former Chief IDB, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Viral and Molecular Pathogenesis

## SECTION

Neural and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

- \* This project was transferred to the LVMP on 10/88 from ID  
\*\* Project was terminated on 10/1/88 due to the departure of the P.I.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS-00402-33 LVMP\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*\*

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Perinatal Infections Causing Damage to the Children in the CPP

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: John L. Sever, M.D., Ph.D. Former Chief IDB, DIR, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Viral and Molecular Pathogenesis

## SECTION

Neural and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

\* This project was transferred to the LVMP 10/88 from ID

\*\* Project was terminated on 10/1/88 due to the departure of the P.I.  
The remaining work involved in the project has been subsumed under:  
Z01 NS 02652-05 BFSB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02765-02 LVMP\*

PERIOD COVERED

October 1, 1988 through September 30, 1989\*\*

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Myelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator. (Name, title, laboratory, and institute affiliation)

PI: L. Hudson, Ph.D. Senior Staff Fellow

LVMP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

\* Project was transferred to LVMP on 10/88 from LMG.

\*\* Project was terminated on 10/1/88; the remaining work involved in this project has been subsumed under Z01 NS 02528-08 LVMP.



TAB 7 -- LABORATORY OF NEURAL CONTROL -- (LNLG)





# ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Neural Control, Basic Neurosciences Program, Division of Intramural Research  
National Institute of Neurological Disorders and Stroke

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Neural Control, Basic Neurosciences Program, Division of Intramural Research  
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

### Introduction

Research work in the Laboratory of Neural Control (LNLC) is devoted to studies of the central and peripheral neural mechanisms involved in the control of movement in mammals, emphasizing neural organizations at the level of the spinal cord and those regions of the brain stem and cerebral cortex that project directly to the spinal cord.

### Present Organization

During FY 1989, the staff of the Laboratory of Neural Control (LNLC) included: eleven professional scientists (five permanent senior scientists, one special expert, and five post-doctoral fellows). The permanent staff also includes eight full-time permanent support personnel (a physiologist, two engineers, one computer programmer, one biological technician, two histology technicians, and one laboratory secretary). Non-permanent staff includes two Fogarty Visiting Fellows, one graduate student, one biological technician, one engineering aide, and one laboratory aide.

The research program of LNLC has undergone a dramatic change in FY 1989 with the arrival of Dr. Michael O'Donovan, who has organized a new Section on Developmental Neurobiology, and with the incorporation of the Section on Neuronal Regeneration under Dr. Andrew Zalewski. Dr. Zalewski's Section was previously in the Laboratory of Neuronal Growth and Regeneration.

The FY 1989 research effort in LNLC can be described under six general headings:

1. Electrophysiological and morphological analyses of the cellular physiology and neuronal circuitry operating in the control of movement at the spinal cord level in cats, either anesthetized or unanesthetized animals following acute destruction of the supratentorial brain (decerebrate preparations).
2. Theoretical and computer modeling studies of cellular biophysical and morphological features of individual neurons, information processing in neural networks, and the kinesiological properties of moving limbs.
3. Studies of the discharge properties of individual neurons in the primate motor cortex and supplementary motor area (SMA) during performance of voluntary movements.
4. Studies of the development of the vertebrate spinal cord, including the maturation of cellular electrophysiological and morphological characteristics, the formation of specific synaptic connections, and analysis of the maturation of intrinsic motor patterns in the chick embryo spinal cord.
5. Studies of the mechanisms of injury repair in mammalian peripheral nerves following axotomy.
6. Activities concerned directly with the development of new methods for making contact with the central nervous system. An important aspect of this during FY 1989 was the initiation of a project to develop a functional prosthesis for stimulation of the human visual cortex. Work also includes the development of instruments and techniques, and the further refinement of existing methods, for recording and analyzing neurally-relevant data.

### Project Summaries

Motor Control Systems in the Cat Spinal Cord: This project utilizes cats, either anesthetized or after decerebration, with destruction of the supratentorial brain. The cat has been used as the ideal model

system for work on the anatomy and physiology of the spinal cord for over a century and there is thus a wealth of detailed information about the cat spinal cord that serves as the basis for the design and interpretation of new experiments. In addition, cats are relatively accessible, have the ideal body size for neurophysiological experiments, and they are well adapted to the laboratory environment. The neural mechanisms that control movement are inferred from data obtained in reduced, immobile preparations. When survival surgery is required, as in studies of the morphology of motor nuclei using retrograde transport methods, surgery is performed under anesthesia and aseptic conditions, and appropriate postoperative care is supplied.

The main goal of these projects is to examine the organization of neuronal systems in the mammalian spinal cord that are involved in the control of movement. Specific topics range from studies of cellular properties of individual neurons in identified neuronal systems to examination of the organization and function of specific neuronal circuits that are involved in movement control, including the problem of central pattern generation at spinal segmental levels.

During FY 1989, we continued our work on the organization of excitatory interneurons that project directly to motoneurons in the cat spinal cord, with emphasis on the input pathways from distal areas of hindlimb skin. We showed earlier that cutaneous afferents in the superficial peroneal nerve (SP) with low electrical thresholds (less than  $2 \times T$ ) produce excitatory postsynaptic potentials (EPSPs) in certain motor nuclei, particularly in flexor digitorum longus (FDL) alpha-motoneurons, often with central latencies consistent with disynaptic connection (less than 1.8 msec). This fact is important because, in principle, interneurons in disynaptic pathways can be functionally identified by virtue of receiving defined, accessible inputs (e.g., particular species of primary afferents or supraspinal descending fiber systems) and by their projection to functionally defined species of neurons, in this case, alpha-motoneurons. We also showed that the initial excitatory components in SP EPSPs in FDL motoneurons are strongly facilitated during the early flexion phase of fictive locomotion, which is the production of rhythmic patterned motoneuron discharges in decerebrate animals that mimic those found in intact, freely walking animals. This indicates that excitatory spinal interneurons, including those in the disynaptic SP to FDL pathway, receive convergent excitation from the central pattern generator (CPG) for locomotion.

During FY 1989, we have used the spatial facilitation technique to show that short-latency EPSPs produced in FDL motoneurons by stimulation of cutaneous afferents in the plantar nerve are also modulated by the locomotor CPG. However, in this case, the plantar EPSPs are markedly reduced in amplitude during the flexion phase, with maximum reduction during early flexion. Thus, the pattern of CPG modulation is for the most part out of phase with that of SP EPSPs. This indicates that there are two quite separate and distinct short latency pathways from low threshold afferents that innervate the dorsal versus ventral surfaces of the toes (SP and plantar nerves, respectively) that are under separate control by the CPG. The skin territories supplied by these two nerves are very close together, yet the pathways that convey information from them to FDL motoneurons are separately controlled during locomotion. The functional meaning of this observation is unclear but it is possible to speculate that the plantar pathway may play a role in the generation of "facultative" FDL activity, which occurs in intact cats during perturbed step cycles and is peculiar to the FDL muscle. Preliminary evidence indicates that PSPs produced in other species of hindlimb motoneurons from the plantar nerve exhibit still other patterns of CPG modulation. Whatever the functional meaning of such differences, they indicate clearly the complexity and finesse of afferent input system control by the locomotor CPG.

We have initiated in FY 1989 a series of experiments designed to locate and identify individual excitatory last-order interneurons in the pathway from SP and plantar nerve afferents to motoneurons. Such identification requires that individual interneurons receive monosynaptic excitation from particular species of skin afferents and project directly to alpha-motoneurons. In a few cases (5 out of over 200 interneurons studied to date), we have been able to show that interneurons recorded in laminae IV - VI receive such monosynaptic input from low threshold mechanoreceptors traveling in the SP nerve and produce monosynaptic EPSPs in L6 motoneurons. The latter criterion is based on recordings using the sucrose gap technique, applied to individual filaments of the L6 ventral root. Although such recordings cannot be used to identify the motor nuclei that receive monosynaptic EPSPs, they provide a practical initial method to locate last order interneurons. We will continue this study, despite its technical difficulty,



in order to examine whether last order excitatory interneurons in the SP cutaneous pathway can be driven by the locomotor CPG in the absence of peripheral input. If so, this would confirm our working hypothesis that such cells may distribute, in whole or in part, excitatory outflow from the segmental CPG.

**Theoretical Studies:** We have used our morphological data base from horseradish peroxidase (HRP)-labeled, type-identified alpha-motoneurons in order to evaluate new approaches to quantitative descriptions of dendritic shape and size. In the past, we and others have described neuronal dendrites by listing quantitative measurements that, while useful, convey no unified notion of dendritic structure. During FY 1989, we began a study of a quite different approach to this difficult problem. Analysis of 1835 dendritic branches from six fully reconstructed motoneurons showed systematic relations between dendritic branch diameter and length, which differed depending on whether the branches ended in a branch point or a termination. The data suggest a strong dependence between branch diameter and the likelihood of branching or terminating. We have found that a relatively simple computer simulation, in which the probabilities of branching or terminating for successive additions of short branch lengths ( $\Delta l$ ) are dependent on local branch diameter alone, produce reasonable fits to the data from actual motoneurons. There was surprisingly little dependence of these probabilities on the length of branches or on the position of the branch within the dendritic tree (e.g., measured as branch order). Analytical and Monte Carlo simulations produced equivalent results. This study suggests that a simple factor related to local branch diameter, possibly the local organization of the cytoskeleton, is the primary agent which maintains, and possibly generates, dendritic structure in alpha-motoneurons.

We have also utilized a variety of approaches, including the new probabilistic model, to analyze the detailed morphology of the dendrites of identified gamma-motoneurons filled with HRP. Although these cells are considerably smaller and simpler in structure than alpha-motoneurons, their dendrites apparently obey quite similar morphological rules. With minor modifications, our probabilistic model generates dendritic branches that fit the data from gamma-motoneurons.

In another subproject, we have utilized our extensive morphological data base for computer model studies of the influence of dendritic location on the shape and amplitude of synaptic potentials recorded at the motoneuron soma. This is a continuation of a long-term study of this problem, which continues to evolve as new questions arise. We have completed a study of the effect of variable dendritic location of group Ia synapses on the detection of single bouton ("quantal") EPSPs using statistical analysis of intracellular potentials. The results suggest that some of the conclusions available in the current literature from such studies, which have neglected this factor, may be in error.

**Cortical Mechanisms of Voluntary Motor Control:** Work in this project is designed to increase our understanding of the organization of neuronal systems in regions of the cerebral cortex, primarily the sensorimotor cortex and supplementary motor area (SMA), of primates that are associated with the control of voluntary movement and which project directly to the spinal cord and brain stem. The major emphasis is on control of voluntary arm, wrist, and finger movements. Most of this research is done using non-human primates (rhesus monkeys), which are intensively trained to perform specific tasks while recording from individual cortical neurons mounted on chronically implanted chambers.

Primates are used for this research because they exhibit the same sort of fine control of hand and individual finger movement present in humans and they can be trained to perform fine hand and finger movements to receive desirable food rewards. Macaque monkeys are used for this work because they readily adapt to the laboratory situation and have been used extensively for earlier work on the hand-arm control by the motor cortex. The new data obtained can thus be readily integrated with the large body of existing data on macaque neuroanatomy and motor cortex physiology. LNLC has pioneered several new methods for ensuring the health and safety of laboratory primates in the experimental situation.

During FY 1989, we have continued studies of the influence of the SMA on movement performance and the activity patterns of individual neurons in the primary motor cortex (area 4) of monkeys. The function of SMA was altered in a reversible manner by cooling it to below 25 degrees C, using a chronically implanted cooling probe. To date, we have found no evidence that alterations in SMA function produce any change in sensory responses in area 4 neurons. There were some alterations in the firing pattern of



approximately 75 percent of task-related area 4 neurons during SMA cooling, with reduction of premovement bursts and increase in average firing during the hold phase of voluntary movement. However, bilateral SMA cooling produced no detectable changes in movement performance or in the response of cortical cells to perturbations introduced during the trained movement. Experiments were devoted to improving the design of the cooling probe to provide more specific unilateral SMA cooling. Thus far, unilateral SMA cooling produce fewer detectable changes than bilateral. These results do not support existing hypotheses that SMA regulates the sensory responsiveness of area 4 cortical neurons in primates.

Our experimental design permits recording of closely spaced pairs of neurons from individual microelectrodes during voluntary movements. Such data are of great interest because they constrain ideas about the local circuit interactions that may occur within the same cortical column. Of the 42 pairs of cells analyzed thus far in which at least one member of the pair fired in relation to the motor task, about one fourth of the pairs exhibited similar firing patterns, another fourth showed reciprocal changes, and one third showed more complex, but related, firing changes. In only 14 percent, there was no relation between the firing patterns of paired cells. These data suggest that neurons in the same or immediately adjacent cortical layers within a cortical column are functionally interconnected in ways that transcend simple equivalence. Presumably, there is a significant amount of local information processing within a cortical column during voluntary movement performance.

**Network Function in Developing Spinal Cord of the Chick Embryo:** This is a new project begun in FY 1989, under the direction of Dr. Michael J. O'Donovan. The primary aim is an analysis of the cellular and network properties during development of spinal neuronal networks that generate the spontaneous motor activity exhibited by chick embryos in-ovo. The work is done entirely with isolated spinal cord preparations in vitro.

A major focus of work during FY 1989 has been on the possible participation of recurrent inhibition in the generation and control of spontaneous rhythmic motoneuron activity and on development of novel optical and electrophysiological recording techniques to circumvent the technical problems of intracellular recording from very small embryonic neurons. We have obtained evidence that recurrent inhibition qualitatively similar to that observed in mammals is present in the chick embryo. However, experiments with pharmacological and activity-dependent blockade of recurrent inhibition suggest that other sources of inhibition appear to be critical in the control the phasing of rhythmic motor activity in embryos.

Experiments that utilize fluorescent calcium-indicator dyes, such as Fura-2 AM and Fluo-3 AM, have been quite successful. Cells can be loaded with these dyes by superfusion, or in some case intracellular injection, and they develop sufficient fluorescence changes even during single action potentials to indicate that there are significant calcium transients in chick embryo motoneurons during single action potentials. Visualization of ensembles of active neurons with calcium and, potentially, voltage-sensitive dyes has great promise in the analysis of patterned motor output, which involves ensembles of interconnected neurons. When particular neurons can be identified as participants in a given motor output pattern, they can be recorded individually using electrophysiological methods.

In addition to optical recording methods, we have found it possible to apply the powerful technique of patch clamp recording to the very small neurons in early chick embryos (12-14 day old) in slice preparations in vitro, following gentle enzyme treatment. Success with patch clamp recording in slice preparations from the CNS is rare and it holds great promise for the study of the development of intrinsic membrane properties in spinal neurons during early development. It seems clear that rhythmic motor patterns in the embryo depend on intrinsic membrane properties as well as neural circuit organization. Continued refinement of the patch clamp approach will be used to complement the studies of circuit organization described above.

An additional project in the Section on Developmental Neurobiology concerns an attempt to develop cytochemical and immunocytochemical markers for particular species of primary afferent fibers - initially to distinguish cutaneous from muscle afferents in chick embryos. The major goal of this project is to determine whether individual afferents are specified as to sensory field before they undergo axonal

outgrowth, and whether their terminal fields within the central nervous system are similarly pre-specified. A new project in this area concerns the cellular events that take place as axonal outgrowth begins. This project uses chick embryo dorsal root ganglion neurons grown at low density in tissue culture, with study of organelle formation and migration using high resolution optical microscopy and video enhancement techniques. Both of these projects are in an early stage of development.

**Mechanisms of Repair Following Injury to Peripheral Nerves:** Work on this project has been ongoing for several years but it represents another new departure for LNLN, which absorbed the Section on Neuronal Regeneration from the Laboratory of Neuronal Growth and Regeneration (LNGR) at the beginning of FY 1989. The major focus of this work is to elucidate the mechanisms by which peripheral nerves are repaired following injury. One aspect of recent work concerns the maturation of the natural barriers that exist in peripheral nerves at the perineurial surface (perineurial barrier) and at the endothelial surface of intraneural blood vessels (blood-nerve barrier). These barriers behave differently during nerve repair and they mature at different rates in newborn to adult rats. At birth, neither barrier is demonstrable using horseradish peroxidase as the large molecule tracer. By 14 days of age, the perineurial barrier is established (HRP cannot enter the nerve from the surrounding tissue) but the blood-nerve barrier at the capillary endothelium is not fully mature until considerably later, at one to two months of age. Enzyme markers thought to be associated with formation of the more extensively studied blood-brain barrier are either not present (gamma glutamyl transpeptidase) or temporally uncorrelated (alkaline phosphatase) with the appearance of the barriers in peripheral nerve.

Additional studies are underway to elucidate cellular events taking place within nerve bundles regenerating through silicone rubber tubes. Of interest is the observation that apparently aneural tissue can form cables within such tubes even when no axons are present. Work is underway to characterize the constituents of the tissue in such cables and to examine the role of each in promoting axonal regrowth. The utility of immunosuppression in using xenografts of peripheral nerve to effect repair is also under test.

**Techniques for Making Contact with the Nervous System:** This project includes all LNLN activities related to the development, design, and fabrication of instrumentation, specialized mechanical equipment, and transducer devices used to support the research work of LNLN, as well as the development of computer software necessary to handle multiple simultaneous streams of data from on-line experiments. Virtually all staff members of LNLN participate in one or more aspects of this project, both as an adjunct to their own research and as a way to share the fruits of their efforts with other staff members and projects. Many of the techniques and instruments developed in LNLN are new and without commercial counterpart. In such cases, LNLN staff continue to provide assistance to other scientists at NIH and at other institutions around the world who request information and advice about specific data acquisition and processing problems.

A major emphasis in FY 1989 has been in the evolution of work to develop a functional and practical visual prosthesis to aid blind patients. Our working hypothesis is that intracortical microstimulation, using chronically implanted "hatpin" electrodes that have been developed over the years in LNLN, provides the safest and most practical approach to chronic stimulation of the human visual cortex in a prosthetic application. Preliminary work described in previous Annual Reports suggested that intracortical microstimulation produces perception of points of light, called "phosphenes", in the visual field of conscious human subjects. In collaboration with the Surgical Neurology Branch (SNB), DIR, NINDS, and the Fundamental Neurosciences Program, NINDS, we have designed a research protocol designed to test this approach with blind human volunteers. The protocol for the human study has been approved and a blind volunteer is being recruited for the study.

In preparation for the human experiment, we have implanted an array similar to that designed for human implant in the visual cortex of a rhesus monkey. We have utilized the NIH facility for magnetic resonance imaging to provide preoperative information of the detailed anatomy of the visual cortex, in order to maximize the chance of proper placement of the electrode array. The animal is currently under study to assess the durability of the individual electrodes during standardized stimulation protocols. Depending on these results, a second animal may be implanted before any human work is undertaken. If

the initial success obtained thus far continues, we anticipate that the first human implant will be done during FY 1990.

We are collaborating with the SNB on a second project designed to test whether pituitary glands contain cells that can produce action potentials. Intra-operative microelectrode recording from two patients undergoing craniotomy under anesthesia for removal of pituitary adenomas have thus far failed to reveal action potentials. It is unclear whether this failure was related to presence of anesthetic and this work will continue during FY 1990, as appropriate patients become available.

Neuromuscular Coordination of Movement: Intramural effort on this project was terminated at the end of FY 1988 but the work has continued during FY 1989 through a research contract with the Department of Electrical Engineering at the University of Maryland (Contract N01-NS-3-2348). Analysis of experimental data collected in LNLN has continued in the Division of Biomedical Engineering at Queen's University, Kingston, Canada under the direction of Dr. Gerald E. Loeb, who directed the project while a staff member of LNLN. Certain data analysis equipment was loaned to Dr. Loeb by LNLN and NINDS during FY 1989 in order to facilitate this process.

The main approach has been to utilize novel methods to study motor performance in intact, behaving cats, as well as the detailed morphological and mechanical characteristics of individual cat hindlimb muscles in order to provide a detailed description of the kinetic system used by the animal during movement. These data are then compared with the predictions made by several theoretical model systems that embody general control principles. Steady progress has been made in both areas. The group at the University of Maryland has integrated their control system equations with a data base system to store experimental results to produce a computer program that allows flexible analysis and display of the kinetics of the whole limb and its relation to the forces and activity patterns of individual muscles. The results to date indicate the necessity of certain control loops within the central nervous system that correspond to known muscle afferent and other reflex organizations but, in addition, predict other relations that are not well recognized. In principle, this modeling approach should provide directions for searching for new spinal cord control systems that require special conditions for demonstration.

The research contract between LNLN and the University of Maryland will terminate in FY 1990.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01686-21 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Motor Control Systems in the Spinal Cord

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                               |                 |             |
|---------|-------------------------------|-----------------|-------------|
| PI:     | R.E. Burke, M.D.              | Chief           | LNLC, NINDS |
| Others: | A.K. Moschovakis, M.D., Ph.D. | Staff Fellow    | LNLC, NINDS |
|         | G.N. Sholomenko, Ph.D.        | Visiting Fellow | LNLC, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Neural Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.55

## PROFESSIONAL:

1.80

## OTHER:

0.75

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is designed to provide information about the structure and function of neuronal mechanisms in the mammalian spinal cord which produce and control movement. These mechanisms include reflex pathways that convey sensory information from primary afferents to alpha motoneurons, interactions between different reflex pathways, modulation of information flow through reflex pathways by supraspinal descending systems, and the production of autonomous rhythmic activity by central pattern generators within the spinal cord. Electrophysiological, neuroanatomical, and computer modeling approaches are used. Recent work has emphasized examination of the modulation of transmission through excitatory cutaneous reflex pathways by the spinal central pattern generator for locomotion in order to clarify the organization of spinal interneurons that control the basic patterning of muscle activation during locomotion in the cat.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01687-21 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Techniques for Making Connections with the Nervous and Musculoskeletal Systems

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                           |                        |             |
|---------------------------|------------------------|-------------|
| PI: M.J. Bak              | Electronics Engineer   | LNLC, NINDS |
| Others: R.E. Burke, M.D.  | Chief                  | LNLC, NINDS |
| G.M. Dold                 | Engineering Technician | LNLC, NINDS |
| M.J. O'Donovan, M.B.Ch.B. | Visiting Scientist     | LNLC, NINDS |
| E.M. Schmidt, Ph.D.       | Biological Engineer    | LNLC, NINDS |
| W.J. Yee                  | Biological Engineer    | LNLC, NINDS |

## COOPERATING UNITS (if any)

Fundamental Neurosciences Program, NINDS (F.T. Hambrecht)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.10

## PROFESSIONAL:

0.20

## OTHER:

0.90

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations. Because of this laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable mechanical transducers, catheters, and connectors. Also included is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01688-21 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cortical Mechanisms of Voluntary Motor Control

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                                   |             |
|---------|----------------------|-----------------------------------|-------------|
| PI:     | E.M. Schmidt, Ph.D.  | Biological Engineer               | LNLC, NINDS |
| Others: | M.J. Bak             | Electronics Engineer              | LNLC, NINDS |
|         | G.M. Dold            | Engineering Technician            | LNLC, NINDS |
|         | F.T. Hambrecht, M.D. | Director, Neuroprosthesis Program | FNP, NINDS  |
|         | C. Kufta, M.D.       | Neurosurgeon                      | SNB, NINDS  |
|         | J.S. McIntosh        | Physiologist                      | LNLC, NINDS |
|         | M. Pomerantz         | Psychologist                      | LNLC, NINDS |
|         | M. Blair             | Psychologist                      | LNLC, NINDS |

## COOPERATING UNITS (if any)

Fundamental Neurosciences Program, NINDS (F.T. Hambrecht); Neuroprosthesis Research Program, NINDS; Univ. Western Ontario, London, Ontario, Canada (Dr. J. Girvin); Surgical Neurology Branch (Dr. Kufta, Dr. Oldfield)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Neural Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.50

## PROFESSIONAL:

0.80

## OTHER:

1.70

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to investigate the spatial distribution and functional properties of cortical neuron 'colonies' in the primate motor cortex that project to the spinal cord and are associated with individual muscles or closely related groups of muscles, as well as the activity of neurons in such colonies during defined voluntary motor behaviors. Cortical cell discharge patterns during normal movements are evaluated in terms of EMG patterns, and their responses to small loading and unloading torque perturbations. These responses are evaluated before, during and after cooling of the supplementary motor area. Spinal cord location of motoneurons innervating selected forelimb muscles and termination patterns in the spinal cord and brain stem of sensory receptors within these muscles are studied using anterograde and retrograde tracing methods.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02079-16 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models of Neurophysiological Systems

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W.B. Marks, Ph.D.

Research Physiologist

LNLC, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Neural Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.40

## PROFESSIONAL:

1.00

## OTHER:

0.40

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As quantitative data become available for a particular form or function in the nervous system, it is advisable to attempt to assimilate the information into a comprehensive model of the underlying mechanisms and their interactions. This project consists in the development of such models and the necessary analytical and mathematical techniques for their implementation and testing in several areas of experimental investigation by LNLC members and other laboratories.

Dr Thomas Smith and Dr. Marks, using the fractal analysis of neurons developed last year, found that the fractal dimension of the shape of optic tract glia growing in tissue culture follow a time course characteristic of the two cell types. Dr. David Lange and Dr. Marks showed that the fourier analysis of neuronal shape can be used to approximate neuronal shape in a very information efficient manner. Dr Burke and Dr. Marks discovered that the dendrites of motoneurons branch and terminate probabilistically at rates that depend only on the local diameter of the dendrite.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02080-16 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuromuscular Coordination of Movement

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                       |                           |             |
|---------|-----------------------|---------------------------|-------------|
| PI:     | G. E. Loeb, M.D.      | Medical Officer, Research | LNLC, NINDS |
| Others: | J.B. Blaszczyk, Ph.D. | Visiting Fellow           | LNLC, NINDS |
|         | C.A. Chanaud          | Guest Researcher          | LNLC, NINDS |
|         | C.J. Heckman          | Guest Researcher          | LNLC, NINDS |
|         | C.A. Pratt, Ph.D.     | Staff Fellow              | LNLC, NINDS |

## COOPERATING UNITS (if any)

Queen's University, Dept. of Physiology, Kingston, Ontario, Canada (F.J. Richmond); U. of Maryland, Dept. of Electrical Engineering

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

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| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project was terminated due to the departure of the Principal Investigator.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02160-15 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intrinsic Properties of Motor Units

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                               |                       |             |
|---------|-------------------------------|-----------------------|-------------|
| PI:     | R.E. Burke, M.D.              | Chief, LNLC           | LNLC, NINDS |
| Others: | W.B. Marks, Ph.D.             | Research Physiologist | LNLC, NINDS |
|         | A.K. Moschovakis, M.D., Ph.D. | Staff Fellow          | LNLC, NINDS |

## COOPERATING UNITS (if any)

(1) Mathematics Research Branch, NIDDK

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Neural Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.30

## PROFESSIONAL:

0.70

## OTHER:

0.60

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project designed to provide information on the electrophysiological and morphological properties of alpha motoneurons and of the muscle fibers (muscle units) innervated by them. The properties of gamma motoneurons are also being investigated. Methods include electrophysiological techniques of intra- and extracellular recording, mechanical recording of muscle unit properties, neuroanatomical techniques of intracellular injection of horseradish peroxidase to label individual, functionally identified motoneurons, retrograde transport of lectin-conjugated horseradish peroxidase to label motor nuclei, and computer modeling studies to analyze the experimental data produced. Recent work has emphasized detailed analysis of the dendritic morphology of alpha- and gamma-motoneurons, with development of a computer model to describe the structure of these dendrites using a minimum number of parameters.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02254-13 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Repair of Injured Nervous Tissue with Foreign Grafts

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                      |             |
|---------|----------------------|----------------------|-------------|
| PI:     | A. A. Zalewski, M.D. | Medical Officer      | LNLC, NINDS |
| Others: | N. A. Azzam, Ph.D.   | Special Expert       | LNLC, NINDS |
|         | R. N. Azzam          | Histopathology Tech. | LNLC, NINDS |
|         | J.D. Ziemnowicz      | Bio. Lab. Tech.      | LNLC, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Neuronal Regeneration

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

4.20

## PROFESSIONAL:

2.00

## OTHER:

2.20

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A short gap in a peripheral nerve can be repaired with a living nerve graft, dead tissue that contains basement membrane tubes (e.g., a frozen nerve graft) or an artificial tube (e.g., a silicone tube) that is initially filled with a fibrin gel. We are analyzing these methods of repair in rats using electron microscopical and histochemical techniques to ascertain how regeneration occurs in each and whether all aspects of nerve function are restored. The perineurial-nerve barrier (PNB) and the blood-nerve barrier (BNB) regulate the movement of macromolecules into the endoneurium from around the nerve and from endoneurial blood vessels respectively. In previous studies, the PNB and BNB were restored in living nerve grafts, whereas, in nerve segments formed in silicone tubes, the PNB but not the BNB developed. To better understand barrier formation, we performed a developmental study using the barrier tracer horseradish peroxidase (HRP). The results indicated that the nerve barriers matured at different times. The PNB kept HRP out of the endoneurium by 2 weeks, a time it leaked out of the BNB (i.e., from endoneurial blood vessels). The BNB did not retain intravenously injected HRP until 6-8 weeks postnatally. In contrast to the blood-brain barrier in which certain enzymes appear, the endothelial cells of the endoneurial blood vessels did not develop any gamma glutamyl transpeptidase activity, and alkaline phosphatase appeared long before the BNB became intact. In an analysis of cellular events occurring in silicone tubes, we found that the cable within it could form in the absence of axons present in the proximal nerve stump. Indeed, this type of cable could support axonal growth through it later on, after a normal nerve end was joined to it. Because the nerve segment formed in a tube is not morphologically normal, we injured it to determine whether this cable would undergo Wallerian degeneration and support axonal regeneration again. As expected, a crush injury of an axonal containing cable formed at 4 months paralyzed the leg of the rat. However, after 8 weeks, the leg recovered movement, and the nerve cable contained regenerated axons in various stages of remyelination.

\* This project was transferred from LNGR to LNLC in October, 1988.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02787-01 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Network Function in the Developing Spinal Cord of the Chick Embryo.

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.J. O'Donovan, M.B.Ch.B.

Visiting Scientist

LNLC, NINDS

Other: E. Sernagor, Ph.D.

Visiting Fellow

LNLC, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Developmental Neurobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

4.45

## PROFESSIONAL:

2.75

## OTHER:

1.70

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is concerned with analyzing the development and function of spinal networks in the lumbosacral cord of the chick embryo. One focus of the study is on the synaptic organization of motoneurons with particular emphasis on sensorimotor and recurrent pathways. A second interest is in analyzing the cellular and network mechanisms responsible for the genesis of spontaneous motor activity. All experiments are performed on an isolated preparation of the spinal cord which is maintained *in-vitro*.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02788-01 LNLC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Primary Sensory Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. L. Smith, Ph.D.

Senior Staff Fellow

LNLC, NINDS

Other: E. Munro

Biologist

LNLC, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neural Control

## SECTION

Section on Developmental Neurobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.50

## PROFESSIONAL:

1.00

## OTHER:

1.50

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general aim of this project is to understand the developmental mechanisms that determine the physiological properties, branching patterns and synaptic connections of primary sensory neurons. One study focuses on the mechanisms that ensure that the various aspects of a sensory neuron's phenotype are correlated and functionally appropriate. In particular, we are interested in finding out whether sensory neurons in chick embryos are pluripotent at the time of axon outgrowth or if, instead, they are already committed to developing specific physiological properties and innervating particular targets. This problem is being addressed by using immunocytochemical labeling and anatomical tracing techniques to study the development of sensory neurons both in intact chick embryos and *in vitro*. A second study is concerned with the mechanisms responsible for determining the polarity and branching patterns of sensory neurons. We are currently using timelapse videomicroscopy to obtain a detailed view of the stages by which sensory neurons grown *in vitro* form their initial processes. The locations of cytoskeletal elements and organelles in these neurons is determined by direct observation and by fixing the cells and staining them with specific antibodies. By revealing the intracellular organization of neurons during process outgrowth, these observations should provide clues as to the underlying cellular mechanisms.









# ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Neurobiology  
Basic Neurosciences Program  
National Institute of Neurological  
Disorders and Stroke

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Neurobiology, DIR

Basic Neurosciences Program

National Institute of Neurological Disorders and Stroke

Thomas S. Reese, M.D., Chief

The Laboratory of Neurobiology has two Sections, the Section on Structural Cell Biology and the Section on Structural Plasticity. The Section on Structural Cell Biology uses modern structural and biochemical techniques to investigate basic cell biological problems germane to an understanding of the function of nerve cells; the Section on Brain Structural Plasticity applies these and other appropriate approaches directly to problems of both fundamental and clinical importance in the mammalian central nervous system, emphasizing problems related to regeneration and response to injury. Current emphasis of the Section on Structural Cell Biology is on the mechanism of axoplasmic transport, axonal growth, and synaptic function while the Section on Structural Plasticity is investigating factors which promote establishment of blood-brain barrier function and neural connections in neural tissues implanted in the brain.

It is the Section on Structural Cell Biology that discovered the molecular basis of the directed organelle movements underlying fast axoplasmic transport. The translocator protein for fast anterograde transport belongs to a new class of motility proteins that we named kinesin. Kinesin (with ATP) is able to mimic the movements serving fast anterograde transport. Kinesin also occurs in many types of non-neuronal cells and appears to be of general significance in cell motility.

Movement induced by kinesin, however, is in only one direction with respect to microtubule polarity. This direction is away from the neuronal cell body, as determined by observing kinesin-induced movements of latex beads along microtubules made from centrosomes (all centrosomal microtubules like those in the neuron have the same defined polarity). Therefore, kinesin must mediate anterograde axonal transport only. There is a separate retrograde translocator which



we have now characterized and purified. This retrograde translocator is a species of cytoplasmic dynein. The anterograde kinesin-induced movements and the retrograde dynein-induced movements can be independently inhibited, suggesting that they function as separate motors. Since organelles can bind both kinesin and dynein, the next question is how kinesin or dynein activation is programmed on the organelle surface.

Metabolic uncouplers of various classes (eg., DNP, CCCP, valinomycin) uniformly block organelle movements along microtubules *in vitro*, but do not block movements of latex beads induced by kinesin or dynein. Since this block cannot be a direct effect on the translocators; requires an intact membrane; and occurs in the presence of ATP, it appears that an ionic gradient across the organelle membrane is responsible for programming an organelle to go in the anterograde or retrograde direction. The nature of this ionic gradient is now being investigated. The structure and dynamic properties of other biological motors are being studied for comparison with the axonal transport motors; the bacterial flagellar motor in E coli has also been shown to depend on interactions of the flagellar structure with membrane proteins, but these motors are driven by rather than controlled by ionic gradients.

Another new approach has been to analyze the kinesin-induced movements of beads on microtubules with computer-based analyses that permit movements as small as 20nm to be defined. The results suggest that beads follow the protofilaments in microtubules, because the moving beads show a lateral shift that corresponds to the pitch of the microtubule protofilaments. Bead movement is also discontinuous, showing a quantal characteristic with subunits that might correspond to individual kinesin-induced events. Bead movements induced by dynein, in contrast to those induced by kinesin, do not follow individual protofilaments. The work on kinesin has also been extended to a consideration of other organelle proteins that might be involved in kinesin-based motility. Organelles isolated from axons are no longer moved by kinesin when they are stripped of extrinsic proteins by high salt treatment; however, certain fractions of the axonal supernatant proteins will restore the ability of kinesin to move organelles along microtubules.

The function of the microtubule-based transport system *in vivo* is also under further investigation since microtubules do not extend the full length of the axon. Reconstructions of serial section electronmicrographs of vertebrate axons and growth cones were used to show the distribution of organelles contacting individual

microtubules. Organelles contacting microtubules were, judging from the previous work on the squid axon, in transport. It appears that each organelle in the vertebrate axons contacts several microtubules, so it is the continuous microtubule bundles which constitute the transport pathways down the axon. The distribution of organelles was uniform along microtubules in proximal growth cones. However, in the mid-segment of the growth cone the frequency of organelles abruptly dropped, even though the microtubules extended to the ends of the growth cone. Thus, there appear to be discrete loading and unloading zones for axonal transport, perhaps related to the degree of microtubule bundling, along the shafts of microtubules in the growth cone.

A preparation of synapses on cell bodies in the mammalian brain that could be studied with rapid freeze techniques was developed this year. The rostral anteroventral cochlear nucleus (AVCN) of the chinchilla provided a preparation in which neuronal cell bodies and synapses in the CNS can be examined after direct freezing and freeze-substitution of rapidly excised brain stem slices. The four types of synaptic terminal known to be in the AVCN were distinguished and correlated with four types of terminals previously reported after chemical fixation. Since the transmitters for each of the four types of terminals have been specified, the transmitter type could be correlated with the detailed structure of the postsynaptic density in each chemical type of synapse. Two types of filamentous components, short vertical projections from the postsynaptic membrane and long filaments protruding from these projections, comprised the basic structure of the post synaptic density; the sizes and distribution of these components differed specifically in each type of terminal. Thus, the freeze-substitution images have provided new information about structural differences between receptor arrays and associated cytoskeletons at different types of central nervous system synapses.

A project which depends on specialized cryotechniques--direct freezing, cryosectioning, electron microscopic immunocytochemistry, quantitative x-ray microanalysis, and elemental x-ray imaging--has yielded new information on the distribution of calcium in Purkinje cells. Quantitative x-ray microanalysis of the intracellular distribution of chemical elements in molecular layer of the cerebellar cortex has shown that the calcium content of the smooth endoplasmic reticulum (ER) within Purkinje cell dendritic shafts, like the ER of dendritic spines, increases substantially (i.e., from 2.6 to 11.2 mmol/kg dry weight) following activation of parallel

fiber synapses. The amount of calcium sequestered by the dendritic ER is similar to that in spine ER (i.e., 11.2 vs. 6.7 mmol/kg, respectively), suggesting that both types of membranes are components of a larger calcium sequestration system.

Cryosectioning techniques, as well as low-temperature embedding, have proven useful for applying electron microscopic immunocytochemistry and in situ hybridization to the localization of membrane and cytoskeletal proteins and of mRNA in sections of nerve and muscle tissues. In the rat neuromuscular junction, the acetylcholine receptor and the 43 kD protein were found concentrated at the crests of the postsynaptic folds, and coextensive with the subsynaptic density. In contrast, sodium channels and ankyrin were concentrated in the membranes of the troughs. This configuration of postsynaptic membrane domains may exist to facilitate the initiation of the muscle action potential. In addition, the results support the involvement of ankyrin and 43 kD protein in anchoring sodium channels and acetylcholine receptors, respectively.

The results on actively myelinating oligodendrocytes of the central nervous system have demonstrated a specific, restricted, and developmentally regulated localization for the large (72 kD) form of the myelin-associated glycoprotein (MAG). This form of MAG appears to be specifically involved in receptor-mediated uptake of a component(s) from the axolemma or periaxonal space, suggesting a possible mechanism for targeting myelin formation. These studies have also demonstrated three distinct modes of synthesis, transport and assembly for the myelin membrane proteins MAG, myelin basic protein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP).

Direct freezing techniques were also used to investigate the distribution of endothelial vesicles and other intracellular organelles in capillaries that had not been subjected to chemical treatment or fixation. Approximately threefold fewer apparently free vesicles were found in directly frozen endothelial cells, as compared with aldehyde-fixed cells, from capillaries of the eel swim bladder and also from cultured human endothelium. However, ultrathin serial sectioning showed that in both cryofixed and chemically fixed eel capillaries, as in the capillaries of the frog mesentery, virtually all vesicles are in continuity with the plasma membrane. Thus, what had been considered free vesicles turn out to be plasmalemmal invaginations; the relationship of these invaginations to endothelial transport, however, remains unclear.

Membrane renewal is an important feature of homeostasis in nondividing cells such as neurons and is especially prominent in the light-receptive membranes of photoreceptors which is, therefore, a suitable model system for study of this phenomenon. Turnover of the photoreceptive membranes was studied in *Limulus* photoreceptors maintained in vitro. These experiments have shown that there are two classes of ventral photoreceptors, which differ in size, morphology, and rhabdom renewal. Rapid, light-stimulated turnover of the photoreceptive membranes was observed in the large photoreceptors, but not in small photoreceptors. Experiments designed to examine the role of the cytoskeleton in rhabdom turnover showed that light exposure reduced the numbers of phalloidin-labeled actin filaments in the rhabdoms of large, but not small photoreceptors.

The Section on Brain Structural Plasticity has continued its exploration of a cell line as a substitute for deficient or absent neurons in the brain. The PC12 (pheochromocytoma) line has been selected because much is known about its functional properties in the naive or neoplastic state and in the neuron-like state induced by treatment with nerve growth factor (NGF). The neuronal condition is also expressed after infection with ras-oncogene and persists much longer, perhaps permanently, compared with that brought about by NGF. We have been defining the functional consequences of differentiating PC12 cells with ras by identifying and localizing, immunohistochemically, the metabolic enzymes for neurotransmitters and by measuring the activities of these enzymes biochemically, in comparison with their activities in naive PC12 cells. Naive and differentiated PC12 cells have both cholinergic and catecholaminergic properties. We find that, as with NGF, there is a marked and sustained enhancement of acetylcholinesterase (AChE) and choline acetyl transferase (CAT) activities. In addition to cholinergic stimulation, we have suggestive evidence that tyrosine hydroxylase (TH), while localized to naive PC12 cells and to NGF and ras- differentiated cells, may be depressed, compared with naive cells, because the content of dopamine in the differentiated cells falls by about 50%. Dopamine measurements are to be repeated.

In order to preclude the possibility of tumors developing from the transplanted cells, it may be necessary to get rid of certain PC12 cells that, after treatment with ras-oncogene, are still neoplastic. We must first determine whether the cells had not been infected with ras or whether they had been infected but did not respond to the oncogene. This differentiation awaits immunostaining with an antibody to P 21, of a



protein that is produced by ras-oncogene. Two ways of deleting the neoplastic cells from the colony to be grafted are (A) to introduce a neomycin - resistant vector along with the ras-oncogene, by retroviral infection. The addition of the antibiotic to ras treated cultures should delete the neoplastic, neomycin-sensitive cells while sparing the ras differentiated ones. (B) Another strategy is to conjugate the highly toxic lectin, ricin, with epidermal growth factor (EGF), the receptors to which are present on neoplastic cells, but are down regulated by NGF. Collaborative efforts suggest that EGF receptors are likewise markedly diminished in number by ras. If this depletion is sufficient, the neoplastic cells should be killed while the ras ones are spared.

The ras-PC12 cells persist for at least 8 weeks, well after the naive cells have disappeared. We conclude that differentiation rather than neoplasia is required for survival of PC12 cells *in vivo*. The differentiated cells retain AChE and TH, although their neurites do not extend into the adjacent striatum. It is likely that they would do so if the surrounding neuropil were deafferented by destruction of the substantia nigra.

The interaction of PC12 cells with brain endothelium was also investigated. Human and experimentally induced brain tumors bring about the formation of fenestrae in capillaries that were once continuous and impervious to large, hydrophilic solutes. Such tumors also produce a vascular permeability factor (VPF), assayed by the increase in the permeability of dermal vessels to protein upon the intradermal injection of the VPF. Before they disappear, it is also known that naive, neoplastic PC12 cells, when grafted to the brain of normal, adult rats cause the development of capillary fenestrae, which denote permeability to hydrophilic solutes. We have set out to isolate and characterize such a VPF that may be secreted by PC12 cells. One end point of the VPF's action would be the fine structural depiction of fenestrae. PC12 cells, *in vitro*, are maintained in serum - free medium for 3 - 5 days. The conditioned medium is partially purified by dialysis against ammonium bicarbonate in casing with a 25,000 Dalton cut-off, then applied to a DEAE - sepharose column. The eluent is lyophilized and incorporated in a slow-release capsule which is implanted into the cerebral cortex of a normal, adult rat. A control capsule, containing endothelial growth supplement is inserted into the opposite cerebral hemisphere of the same animal. To date, the single specimen examined did not have fenestrated vessels. If the PC12 cells do secrete a VPF, it must be further purified and concentrated and included in a capsule with a higher rate of release. The fenestrated vessels, like those elsewhere, might be permeable to macromolecules via a tubulo-vesicular system rather than

fenestrae. Accordingly, the permeability of the vessels near the PC12 cells would be tested with intravascularly injected tracer.

Another question was whether brain endothelial cells, (BE), influence the properties of neurons and of PC12 cells. Endothelial cells, dissociated from bovine brains, are co-cultured with primary cultures of fetal and neonate rats or with PC12 cells, differentiated into neuron-like cells with ras-oncogene. The co-cultures are examined with immunostaining, biochemical assays and by electron microscopy. We find that BE cells greatly enhance the survival and growth of neurons in primary culture. About ten fold more neurons survive in co-culture than in solo neuron cultures. Some of the neurites had synaptic vesicles and made synaptic contacts. BE also augmented the number of neurites and small vesicles belonging to naive as well as to NGF and ras-differentiated PC12 cells. Acetylcholinesterase and choline acetyl transferase activities were more than ten fold higher than in naive PC12 cells. Tyrosine hydroxylase activity also increased significantly. Conversely, PC12 cells and cortical neurons, in turn, brought about some morphological changes in endothelial cells: greatly distended rough endoplasmic reticulum, abundant, packed actin and copious extracellular matrix. Thus, there is a metabolic and a morphologic, reciprocal, influence of BE and neurons *in vitro*.

In order to further characterize the internal structure of the astrocyte cell membrane, we have extended our previous finding that the presence of brain endothelial cells in co-culture with astrocytes leads to a rise in the number of and an aggregation of orthogonal particle units or assemblies in the astrocyte plasma membrane. We have now found that the "background" particles, scattered among the assemblies, respond highly selectively to incubation, *in vitro*, with 0.01U/ml of phosphatidylcholine - phospholipase C (PC-PLC) from C. perfringens. The responsive cells were normal astrocytes, C6 glioma cells and retinal Muller cells whereas 6 other cell types : brain endothelial, aortic endothelial, PC12, cerebellar granule and fibroblast cells, and a tracheal fibroblast cell line, did not respond even to doses 10 times greater. The response was twofold : (A) The particles reversibly aggregated 2 - 6 h after incubation with the PC-PLC. Cells fixed 24 h after incubation had normally distributed particles. (B) The astrocytes shrunk and the cytoskeleton became sharply compartmentalized: sublemmal actin was most peripheral, followed by microtubule rows and then, with very little overlap of domains, intermediate filaments. The significance of these results is that specific membrane changes in astrocytes were brought about by the cleavage



of phosphatidyl choline within the plasma membrane. The cytoskeletal changes await further confirmation. Others have documented the removal, by phospholipase C, of a lanthanum - infiltrated layer on the outer surface of cells. Whether this action is even more pronounced in astrocytes is to be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-01442-23 LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders )

Permeability of Cellular Layers in the Vertebrate Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |                    |            |
|---------|---------------------------|--------------------|------------|
| PI:     | T.S. Reese, M.D.          | Chief              | LN, NINDS  |
| Others: | S. B. Andrews, Ph.D.      | Research Chemist   | LN, NINDS  |
|         | J. Frokjaer-Jensen, Ph.D. | Guest Researcher   | LN, NINDS  |
|         | B. Kachar, Ph.D.          | Visiting Associate | LNO, NIDCD |

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543. J.S. Handler, KE, IR, NHLBI, NIH, Bethesda, MD. R.P. Rand, Brock University, St. Catherine's, Ontario, Canada. R.C. Wagner, University of Delaware, Newark, DE.

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892. Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The substructure of tight and gap junctions is investigated by direct freezing techniques that avoid any chemical fixation. How tight junctions prevent small charged solutes from entering the brain (across the blood-brain barrier) is made clear by our new model of tight junction structure based on a lipidic backbone. Tight junctions in invertebrates also appear to have lipidic backbones. New structural evidence suggests the presence of periodic supporting structures, presumably proteins, along the lipidic backbones. Direct freezing and freeze-substitution were also used to investigate the three-dimensional organization of the vesicular system in capillaries of the rete mirabile of the eel. Using improved morphometric approaches, it was shown that, regardless of the number of endothelial vesicles present, essentially all vesicles remain interconnected with each other and are part of two separate sets of invaginations from the luminal and abluminal cell surface.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-01881-19 LN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Structural Basis of Synaptic Transmission and Development

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. S. Reese, M.D..

Chief

LN, NINDS

Others: H. Tatsuoka, M.D.

Visiting Scientist

LN, NINDS

T.P.O. Cheng, Ph.D.

Visiting Associate

LN, NINDS

## COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543, -D.M.D. Landis, Case-Western Reserve University, Cleveland, OH.

## LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

## SECTION

Section on Structural Cell Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892. Marine Biological Laboratory, Woods Hole, MA 02543

## TOTAL MAN-YEARS

2.3

## PROFESSIONAL:

1.8

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A preparation of synapses on cell bodies in the mammalian brain that could be studied with rapid freeze techniques was developed this year. The rostral anteroventral cochlear nucleus (AVCN) of the chinchilla has provided a preparation in which neuronal cell bodies and synapses in the CNS can be examined after direct freezing and freeze-substitution of rapidly excised brain stem slices. The four types of synaptic terminal known to be in the AVCN were distinguished and correlated with four types of terminals previously reported after chemical fixation. Since the transmitters for each of the four types of terminals have been specified, the transmitter type could be correlated with the detailed structure of the postsynaptic density in each chemical type of synapse. Two types of filamentous components, short vertical projections from the postsynaptic membrane and long filaments protruding from these projections, comprised the basic structure of the post synaptic density; the sizes and distribution of these components differed specifically in each type of terminal. Thus, the freeze-substitution images have provided new information about structural differences between receptor arrays and associated cytoskeletons at different types of central nervous system synapses. Work on the cultured growth cones continues and is now concentrated on developing instrumentation in order to rapid freeze growth cones while they are being observed by DIC microscopy.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02551-08LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Structure of Neuronal Cytoplasm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                     |                    |           |
|---------|---------------------|--------------------|-----------|
| PI:     | T.S. Reese, M.D.    | Chief              | LN, NINDS |
| Others: | T.P.O. Cheng, Ph.D. | Visiting Associate | LN, NINDS |
|         | P. Gallant, Ph.D.   | Special Expert     | LN, NINDS |
|         | B.J. Schnapp, Ph.D. | Staff Fellow       | LN, NINDS |
|         | M. Terasaki, Ph.D.  | Staff Fellow       | LN, NINDS |

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543. M. Sheetz, Washington University, St Louis, Mo. T. Schroer, Washington University, St. Louis, Mo. R. Vale, Ph.D. University of California, San Francisco, CA. B. Kachar, National Institute of Health, Bethesda, MD

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892. Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project determines the structure of neuronal and glial cytoplasm, particularly as it pertains to axoplasmic transport. A protein translocator, kinesin, responsible for the anterograde organelle movements along microtubules which are the basis of anterograde fast axonal transport, has been characterized. We have now purified from the axoplasm of squid giant axons a high molecular weight protein, which we have characterized as a cytoplasmic dynein, which transports exclusively in the retrograde direction. Since organelles can bind both kinesin and dynein, the next question is how kinesin or dynein activation is programmed on the organelle surface. Metabolic uncouplers of various classes uniformly block organelle movements along microtubules in vitro, but do not block movements of latex beads induced by kinesin or dynein. Since this block is not a direct effect on the translocators, requires an intact membrane, and occurs in the presence of ATP, it appears that an ionic gradient across the organelle membrane is responsible for programming an organelle to go in the anterograde or retrograde direction. The function of the transport system in vivo is also under investigation since microtubules do not extend the full length of the axon. It appears that each organelle contacts several microtubules in the axon, so it is the continuous microtubule bundles which constitutes the transport pathways down the axon. Much of the pool of kinesin and dynein is in a soluble form and new immunocytochemical methods are being developed to determine their distributions in cytoplasm in relation to the transport pathways.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02610-06 LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Distribution of Mobile and Structural Components at Chemical Synapses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                            |                    |           |
|---------|----------------------------|--------------------|-----------|
| PI:     | S. Brian Andrews, Ph.D.    | Research Chemist   | LN, NINDS |
| Others: | Thomas S. Reese, M.D.      | Chief              | LN, NINDS |
|         | Asher Shainberg, Ph.D.     | Visiting Scientist | LN, NINDS |
|         | Bernhard E. Flucher, Ph.D. | Visiting Fellow    | LN, NINDS |
|         | Maureen F. O'Connell       | Biologist          | LN, NINDS |

COOPERATING UNITS (if any)

R.D. Leapman, BEIB, DRS, NIH, Bethesda, MD. D.M.D. Landis, Case-Western Reserve University, Cleveland, OH. B.D. Trapp, Johns Hopkins University School of Medicine, Baltimore, MD. M.P. Daniels, LBG, NHLBI, NIH, Bethesda, MD.

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892. Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to determine the intracellular distribution of diffusible and structural components within axons, dendrites, glia, and synapses. Quantitative x-ray microanalysis of the intracellular distribution of chemical elements in Purkinje cell dendritic shafts has shown that some cisterns of smooth endoplasmic reticulum in the dendrites, like the cisterns in dendritic spines, sequester substantial amounts of calcium following activation of parallel fiber synapses. Electron microscopic immunocytochemistry has revealed a striking organization of the postsynaptic membrane of the neuromuscular junction. Acetylcholine receptors and the 43kD protein are co-localized at the crests of postsynaptic folds, while sodium channels and ankyrin have a complementary distribution in the troughs. Immunogold electron microscopy has also been used to show that the 72 kD form of the myelin-associated glycoprotein (MAG) in oligodendrocytes is, in contrast to Schwann cells, specifically enriched in periaxonal membranes and in large multivesicular bodies during early postnatal development only. This suggests that the 72-kD MAG is involved in receptor-mediated endocytosis of components from the periaxonal space or axolemma. Immunocytochemistry and in situ hybridization have further indicated that the locations of the myelin protein 2',3'-cyclic nucleotide 3'-phosphodiesterase and of its mRNA are distinctly different from MAG and myelin basic protein. This implies differential mechanisms of intracellular transport for proteins destined for the myelin sheath.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02700-04 LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Mechanochemistry of Proteins Involved in Axonal Transport

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                       |                     |           |
|---------|-----------------------|---------------------|-----------|
| PI:     | B.J. Schnapp, Ph.D.   | Senior Staff Fellow | LN, NINDS |
| Others: | S.H. Khan, Ph.D.      | Guest Researcher    | LN, NINDS |
|         | Thomas S. Reese, M.D. | Chief               | LN, NINDS |

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543. M.P. Sheetz, Washington University, St. Louis, Mo. J. Gelles, Washington University, St. Louis, Mo. S.H. Kahn, Albert Einstein College of Medicine, Bronx, NY.

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892. Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL MAN-YEARS

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project was to understand how the motors which power fast axonal transport interact with microtubules to promote movement. A second goal arising during the previous year has been to investigate how the motor proteins interact with vesicular organelles to generate movement in either the anterograde or retrograde directions. We have analyzed microtubule gliding and bead movement along microtubules powered by kinesin and by the recently purified retrograde motor which turns out to be dynein. In addition to analyzing the movement of artificial substrates coated with the motor proteins, the motion of organelles purified from axoplasm is being investigated. This project involves analyzing microtubule-based motility by video microscopy, using a digital processor to generate images with sufficient contrast to visualize single microtubules and to acquire and analyze motion as a function of manipulations of the chemical environment. A procedure for tracking at 2 nanometer definition the motion of organelles or beads moving on microtubules has been successfully implemented. This new technique has provided information, which has now been published, about the molecular mechanical events that drive the movement of organelles along microtubules. Since the PI has left NIH, this project is on abeyance until publications are completed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01-NS-01805-21LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Membrane Structure of Astrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                       |                     |           |
|---------|-----------------------|---------------------|-----------|
| PI:     | J.H. Cheng, Ph.D.     | Senior Staff Fellow | LN, NINDS |
| Others: | J.P. Bressler, Ph.D.  | Guest Worker        | LN, NINDS |
|         | M.W. Brightman, Ph.D. | Section Chief       | LN, NINDS |

COOPERATING UNITS (if any)

Kennedy Institute of Mental Health; Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892.

TOTAL MAN-YEARS:

1.5

PROFESSIONAL

1.2

OTHER

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown that phosphatidylcholine phospholipase C from C. perfringens (PC-PLC) caused astroglial membrane particles to aggregate at a concentration of 0.05U/ml for 5 hrs and that this effect is specific to astrocytes. None of 6 other control cell types were similarly affected. We now demonstrate that this effect on astroglial membrane can be blocked by withholding the calcium in the incubation medium or by boiling the enzyme. Both of these conditions are known to block PC-PLC's activity. In addition, thin sections of PC-PLC - treated astrocytes show distinctly compartmentalized cytoskeletal elements, especially at peripheral processes. Actin filaments lay immediately underneath the plasma membrane, while microtubules and intermediate filaments were more centrally situated in their own domains with almost no overlap. This effect of PC-PLC on the astroglial cytoskeleton is probably separate from its effect on the astroglial plasma membrane because the membrane change is reversible after 24 hrs and the cytoskeletal changes are not.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02086-16 LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regeneration Specificity in Transplanted Neural Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                     |                     |            |
|---------|---------------------|---------------------|------------|
| PI:     | O. Okuda, Ph.D.     | Visiting Fellow     | LN, NINDS  |
| Others: | D.L. Simpson, Ph.D. | Special Expert      | LN, NINDS  |
|         | J.H. Cheng, Ph.D.   | Senior Staff Fellow | LN, NINDS  |
|         | J. Bressler, Ph.D.  | Guest Worker        | LN, NINDS  |
|         | G. Guroff, Ph.D.    | Section Chief       | IRP, NICHD |
|         | M. Brightman, Ph.D. | Section Chief       | LN, NINDS  |

COOPERATING UNITS (if any)

NICHD, Division of Intramural Research, NIH  
Kennedy Institute of Mental Health, Johns Hopkins University, Baltimore, M.D.

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892.

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.4

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Neoplastic PC12 cells cease dividing and differentiate into neuron-like cells when they are either continually exposed to nerve growth factor (NGF) or infected with a ras-oncogene. (I) - What are these neuronal properties, how do they differ between NGF-treated PC12 cells and ras-PC12 cells and what are the mechanisms of neuronal expression? Naive, NGF or ras-treated PC12 cells, are maintained in vitro for several weeks. Compared with naive PC12 cells, acetylcholinesterase increases 40 - 50% in ras- and NGF cells. From preliminary data, choline acetyl transferase is 20 - 40% higher in ras- and NGF PC12 cells. Nor-epinephrine (NE) uptake by ras- cells was twice as high in Na<sup>+</sup>-containing medium, while the amount of dopamine (DA) in ras-cells is only 50% that of naive PC12 cells. Thus, ras-differentiation enhances cholinergic activity and NE uptake, but diminishes DA content. (II) - What is the fate of ras-PC12 cells implanted into the brain of normal, adult rats? Neoplastic PC12 cells, grafted into the striatum on one side of the brain, induce the formation of very large hemorrhagic cavities. The PC12 cells disappear by 3-4 weeks after grafting. Implanted ras-PC12 cells survive for at least 8 weeks, express tyrosine hydroxylase, detected immunohistochemically and acetylcholinesterase, detected histochemically. Their neurites are confined to the graft and do not enter the surrounding striatum. Instead of forming tumors, neoplastic PC12 cells die when grafted to adult rats. It is the ras- differentiated cells that survive. We assume that their neurites will extend into adjacent neuropil that has been deafferented by destroying the substantia nigra.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02144-15LN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Blood-Brain Barrier

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

|         |                            |                     |           |
|---------|----------------------------|---------------------|-----------|
| PI:     | J.H. Cheng, Ph.D.          | Senior Staff Fellow | LN, NINDS |
| Others: | David L. Simpson, Ph.D.    | Special Expert      | LN, NINDS |
|         | Shoichiro Ishihara, M.D.   | Visiting Fellow     | LN, NINDS |
|         | Milton W. Brightman, Ph.D. | Section Chief       | LN, NINDS |

COOPERATING UNITS (if any)

M. Merrill, Ph.D. , Surgical Neurology Branch, NINDS

LAB/BRANCH

Laboratory of Neurobiology, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892.

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

1.7

1

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

I - Characterization of a vascular permeability factor (VPF) from PC12 cells. When neoplastic PC12 cells are transplanted to brain, the capillaries near the grafted cells become fenestrated. Such permeable vessels supply human brain tumors. Can a VPF, isolated from culture medium conditioned by PC12 cells, induce fenestrae in brain endothelium? PC12 cells are grown in serum-free medium for 3 to 5 days. The conditioned medium is partially purified and incorporated into a slow-release capsule that is inserted into the cerebral cortex of rats. A capsule containing endothelial growth factor is placed in the opposite hemisphere as a control. In one brain examined by electronmicroscopy 2 weeks after grafting, no fenestrae were found. II - Cellular interactions of brain endothelium and neurons in vitro. Can brain endothelium (BE), which alters membrane structure of astrocytes in vitro, also affect the behavior of neurons? We find that BE markedly enhances the survival and growth of fetal and neonatal cerebral neurons, in primary culture. By means of immunohistochemistry and biochemical assays, we find that BE also induces PC12 cells to produce more choline acetyl transferase, acetylcholinesterase and tyrosine hydroxylase. It is likely that the synthesis of acetylcholine and dopamine are also enhanced. The BE in such co-cultures have distended rough endoplasmic reticulum, abundant actin and produce unusually large amounts of extracellular matrix, detected by electron microscopy. Thus, BE and neurons in co-culture affect each other's growth and differentiation.





# ANNUAL REPORT

October 1, 1988 through September 30, 1989

## Laboratory of Neurochemistry

National Institute of Neurological Disorders and Stroke

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## **ANNUAL REPORT**

October 1, 1988 through September 30, 1989

Laboratory of Neurochemistry, Division of Intramural Research

National Institute of Neurological Disorders and Stroke

Harold Gainer, Ph.D., Chief

The laboratory is concerned with the development and functional organization of the nervous system, with a specific focus on molecular mechanisms. The approach of the laboratory is cellular and molecular biological in nature and utilizes a wide variety of techniques and concepts from a number of disciplines, e.g., physiology, biochemistry, biophysics, morphology, immunology, and molecular biology. Specifically, we study neuropeptides and other neurotransmitters, neuropeptide receptors, plasma membrane and intracellular membrane systems, cytoskeletal proteins, and various enzymes (e.g., Na,K-ATPase, protein kinases, and proteases) which are found in the nervous system and are essential to its development and function.

The Laboratory of Neurochemistry is composed of three Sections: Cellular and Developmental Neurobiology, Enzyme Chemistry, and Molecular Neuroscience.

### I. Section on Cellular and Developmental Neurobiology

Studies on the role of neuropeptides in nervous system development and function, and the molecular mechanisms underlying the unique morphologies of neurons constitute the major activities in this Section. The vertebrate nervous system is organized in a highly complex but ordered array of neuronal networks, in which the individual units (neurons) interact using a large number of chemical and electrical signals. Even at the level of the single neuron, a myriad of chemical messengers (e.g., neurotransmitters, neurohormones, paracrine, autocrine, and trophic factors), afferent inputs, ion channels, receptors, and signal transduction systems are integrated to produce physiologically relevant outcomes. In order to understand the rules that govern the origins and functions of neuronal networks, it is essential to understand the contributions of the above mechanisms to neuronal populations which integrate this vast array of inputs (signals) to generate a decisive output.

In this Section, we have focused on selected neuronal populations in the hypothalamus and peripheral nervous system which we believe are models for the analysis of the regulation of peptidergic phenotype and neuronal morphology. Some

questions we are addressing, using these systems, are: 1) What are the ontogenetic histories of these neuronal populations? How do their cell lineage relationships and migratory patterns during development relate to their differentiated phenotypes (e.g., specific peptide expression)? 2) What are the mechanisms which underlie the establishment of the differentiated neuronal phenotypes? These issues include considerations of heterogeneity of differentiated properties of the populations (subpopulations), their membrane, receptor, and signal transduction systems, unique morphologies and relevant intrinsic cytoskeletal proteins, and axonal outgrowth and nerve terminal distributions. 3) What are the regulatory elements in the genes uniquely expressed by these neurons during development and homeostatically after maturation?, and 4) To what extent do these neuronal populations exhibit "plasticity"?

Most peptidergic neurons in the central nervous system (e.g., oxytocin and vasopressin neurons) derive from precursor cells in the ventricular germinal zone. Recent studies in our Section, however, have demonstrated that luteinizing hormone-releasing hormone (LHRH) neurons normally located in the adult forebrain are in fact derived from progenitor cells outside of the central nervous system proper, i.e., in the olfactory placode, and subsequently migrate along 'tracks' in the nasal area towards the brain. These cells first accumulate at the base of the telencephalon, after which they penetrate the brain and migrate towards their final resting destinations in the forebrain. Current questions relate to the nature of the migration pathway, i.e., which cellular processes (neuronal or glial) form the 'tracks', and which extracellular proteins mark these pathways and migration arrest sites? Although formation of the LHRH system is a prenatal event, its function is primarily postnatal; i.e., occurring after puberty. The analysis of postnatal development and function requires an in vitro model system. Recently, organotypic slice explant cultures have been developed in our Section which allow for the study of LHRH neurons (and other peptidergic neurons, e.g., vasopressin, oxytocin, corticotropin releasing hormone, and thyrotropin releasing hormone-containing neurons). Studies of the regulation of peptide gene expression in these neurons under rigorously controlled environmental circumstances are currently in progress.

A second feature of neuronal phenotype under investigation in our Section is peptide coexistence. In the hypothalamus-neurohypophyseal system, AVP is colocalized with dynorphin, and OT is colocalized with CRH and/or CCK peptides. The biological significance of these co-localizations has been examined by studying the effects of dynorphin, CCK, and CRH on secretion of AVP and OT from the neurohypophysis. Dynorphin peptides were found to selectively inhibit OT secretion, whereas CRH and CCK caused an increased secretion of both AVP and OT. The three peptides were found to work via different mechanisms: the dynorphin inhibition acts by direct action on OT nerve terminal membranes, the CRH by paracrine action on the intermediate lobe to evoke MSH release which feeds back to induce AVP and OT

secretion in the neural lobe, and the CCK to induce secretion by a protein kinase C mediated mechanism in the neural lobe itself. In the corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus, AVP and CRH are normally co-localized in only 50% of the neurons projecting to the median eminence, indicating that there are two separate populations (subpopulations) of CRH neurons, which could be independently regulated. Recent studies have shown that the CRF+AVP+ subpopulation is preferentially activated during stress. A central issue of peptide coexistence is the elucidation of the molecular mechanisms which underlie the various combinations of peptide coexistence in individual neurons. Sensory ganglion neurons exhibit a remarkable degree of peptide coexistence, and studies on the regulation of specific peptide gene expression in these neurons in dissociated cell culture is a major activity in this Section.

In addition to containing neurons which secrete specific peptides, a functioning network must also contain peptide receptors. In our Section, the analysis of peptide receptors has focused on three candidates: the CCK receptor, bombesin-like peptide (Gastrin-releasing peptide) receptor, and the Vasopressin (V1) receptor. Messenger RNAs from cultured cell lines containing these receptors have been injected into Xenopus oocytes, which then express these receptors and are assayed by pharmacological/electrophysiological methods. Efforts to clone these receptors are in progress, using the Xenopus assay for identification of relevant clones (see Section on Molecular Neuroscience summary for more details).

At present, the study of cytoskeletal proteins in relation to neuronal morphology in our Section is restricted to the study of neurofilament proteins. Studies on the post-translational regulation of neurofilament proteins have shown that these proteins, which appear to stabilize axonal structures, are highly phosphorylated. Using monoclonal antibodies and immunocytochemistry we have shown that the higher molecular weight neurofilament proteins (NF-M and NF-H) are principally phosphorylated in axons at later stages of development. A unique neuronal intermediate filament protein in Xenopus has been cloned and sequenced, and it has been localized to peripheral axons. Studies in the squid giant axon system on neurofilament structure and function continue to support our hypothesis that the genesis of neurofilament structure is determined topographically in the neuron, i.e., biosynthesis and assembly in the cell body, phosphorylation in the axon, and proteolysis in the terminal. This topographic organization of regulation of post-translational modification is currently under study with regard to mechanism. We have found that the neurofilament phosphorylation appears to protect the neurofilaments from degradation by calpain (a calcium-dependent protease presumed to act on NFs in nerve terminals), and that both squid axoplasm and bovine spinal cord contain unique protein kinases that can phosphorylate neurofilament proteins. Experiments employing mammalian sensory ganglion explants are currently underway which are investigating the effects of specific protein kinase activations on the phosphorylation of



specific serine and threonine residues (sites) in the three neurofilament protein subunits (NF-L,-M,-H) in cell bodies versus axons. Probably the most significant issue with respect to the neurofilaments is what role they play in neuronal structure and function. The most popular hypothesis at present is that these molecules stabilize axonal structure and are involved in increasing axonal caliber. We are testing this hypothesis by injecting antibodies to specific neurofilament subunits and forms into one blastomere of Xenopus embryos at the two cell stage. With subsequent development these antibodies partition selectively into only cells which derive from the injected blastomere. In Xenopus, this leads to a bilateral difference in the antibody distribution and also appears to modify axonal outgrowth and branching only in those neurons which contain the antibodies.

## II. Section on Enzyme Chemistry

The major projects in the Section on Enzyme Chemistry are concerned with the relationships between structure, function and the mechanism of the ATP-dependent cation transport proteins. The ability of cells to use metabolic energy to create and maintain gradients of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and protons depends directly on these transport proteins. In the case of the sodium-potassium pump, some general principles of the transport mechanism and considerable structural information is now available, but relatively little is known about the relationships between structure and function. Current projects in the Section on Enzyme Chemistry are directed toward 1) resolving certain mechanistic questions and 2) gaining information about the relationships between structure and function.

Rapid quenching kinetic studies: two studies have been completed that utilize a rapid quenching technique to obtain information about the pre-steady state kinetics of the phosphorylation and dephosphorylation reactions that occur at the catalytic site of the sodium-potassium pump protein. The results of these studies support the hypothesis that the functioning of the sodium pump involves oligomeric interactions of the pump subunits.

We have developed a series of antibodies against synthetic peptides that correspond to strategically located segments of sodium pump proteins. We are studying the isoforms of the sodium pump that are expressed in rat brain tissue. All three proteins occur in the adult brain. Studies of the regulation of these isoforms are in progress employing immunological, immunohistochemical and cell culture techniques.

The sodium pump consists of equimolar  $\alpha$  and  $\beta$  subunits. Little is known of the function of the  $\beta$  subunit, which is heavily glycosylated. Although, as yet, there is good evidence for only a single message for the  $\beta$  subunit, the expressed protein displays a

marked degree of microheterogeneity. We have recently shown that much of this, in *Electrophorus electric* organ, can be ascribed to the sialic acid component. Techniques were developed that further characterize the glycosyl groups of the  $\beta$  subunits. We are currently extending these studies to brain tissue.

Regulation of sodium pumps in neural cells is currently being investigated in a collaborative study involving the use of primary cultures of cerebellar neurons. Initial results indicate that both the absolute and relative amounts of the isoforms are sensitive to the culture conditions.

Several aspects of these studies are interrelated. Our recent kinetic studies of the sodium pump from *Electrophorus electric* organ indicate that, even in a preparation that appears to be a single isoform, the kinetics are complicated by oligomeric interactions. This raises questions about cells that express more than one isoform. Do isoforms interact within the same cell to form functional hybrids? Are the  $\beta$  subunits that combine with different isoforms identical? Are these variables functionally significant? Some of the techniques to address such questions may now be at hand.

### III. Section on Molecular Neuroscience

The goal of this Section is to use a molecular approach to explore the structure, function, and regulation of neuropeptide hormone and neuropeptide receptor genes in the mammalian nervous system. The current effort is focused on the mammalian bombesin-like peptides, gastrin releasing peptide (GRP) and neuromedin B (NM-B), and their receptors. The bombesin family of peptides is expressed in many brain nuclei, the dorsal root ganglia, and the posterior spinal cord. They are potent neuropeptides eliciting a variety of central homeostatic and behavioral responses including poikilothermia, regulation of blood glucose levels, anorexia, alterations in gastric acidity, and scratching behavior. These peptides have also been detected in the intrinsic neurons of the gut, where they regulate smooth muscle contraction, stimulate the release of gastrin from G cells of the antral gastric mucosa, and function as secretagogues for a variety of gastroenteropancreatic hormone. In addition, bombesin-like peptides are mitogens for growth-arrested murine embryonal Swiss 3T3 fibroblasts in culture, G cells in neonatal rat antral mucosa, and human pulmonary explants, indicating that under some circumstances these peptides can stimulate mitosis as well as transduce a secretory or neuromodulatory signal.

We and others have obtained cDNA and genomic clones for the prohormones encoding the two known mammalian bombesin-like peptides, GRP and NM-B. The rat preproGRP gene has a three-exon structure encoding a signal peptide, the GRP peptide followed by a glycine alpha-amidation donor and two basic amino acids.



Cleavage at this pair of amino acids followed by amidation of the carboxyl-terminal residue of the GRP peptide releases a biologically active GRP peptide, and also generates an approximately ninety amino acid extension peptide of unknown function. The gene is expressed in many brain nuclei, most prominently in the suprachiasmatic nucleus of the hypothalamus. Two forms of the mRNA are found in brain: a more abundant 1.1 kb form which initiates in both central and peripheral neurons, and a less abundant 1.5 kb mRNA form, whose initiation sites are heterogeneous, located several hundred bases upstream of the 1.1 kb initiation site, and is used only in spinal cord and a subset of brain nuclei expressing preproGRP mRNA. Studies performed on cultured cells expressing the human preproGRP gene indicate that gene regulation occurs primarily at the level of transcription initiation, and involves chromatin structural changes resulting in DNase hypersensitive sites. The molecular mechanisms responsible for cell-type specific regulation of the preproGRP gene are an area of intense interest that we plan to pursue in detail in the future.

We have isolated and characterized cDNA and genomic clones for rat preproNM-B. The gene has a three-exon structure analogous to that described previously for the preproGRP gene, consistent with the view that the two genes diverged from a common ancestral precursor gene. Similarity between the two genes is observed only over the carboxyl ten amino acids of the GRP and NM-B peptides, which is the region of the peptide necessary and sufficient for high affinity binding to bombesin receptors. The sequences on the two genes in the promoter region show no regions of similarity, suggesting that the two genes are independently regulated. Expression of the gene results in a single 1.0 kb mRNA species that is most abundantly expressed in brain and gut. The initiation site in the brain appears to be heterogeneous, and not TATAA-directed. In situ hybridization studies localizing NM-B mRNA in the brain indicate that the distribution of cells and loci expressing NM-B is quite distinct from those expressing GRP, and more limited. Both genes contribute independently to the bombesin-like immunoreactivity described previously in the brain. Neuromedin B mRNA is expressed at high levels in trigeminal and dorsal root ganglia neurons. In collaboration with Dr. Gainer, we are currently exploring the possibility that primary cultures derived from these ganglia may provide a population of neurons expressing the neuromedin B gene. These cells would be appropriate hosts for promoter-reporter fusion gene transfection studies done in collaboration with Dr. Gainer's lab to define transcription regulatory elements in the neuromedin B promoter.

In collaboration with Dr. Kiyoshi Kusano, we are attempting to use a molecular genetic approach to identify a cDNA clone for the bombesin receptor. We have built a size-fractionated subtracted cDNA library which is highly enriched for genes expressed exclusively in receptor-bearing fibroblasts. This collection of cDNA clones will be surveyed to obtain representatives of each gene found in the library. Representative clones will be used to hybrid select mRNA for subsequent injection into

Xenopus oocytes, where expression of the bombesin receptor protein can be monitored as a ligand-dependent chloride current. Currently, we are optimizing our hybrid selection strategy to allow screening of pools of candidate clones. When a positive clone is identified, it will be used as a probe to isolate a full-length cDNA clone for structural analysis. The full-length clone will be the basis for site-specific mutagenesis experiments probing structure-activity relationships in the receptor protein after expression in Xenopus oocytes or in fibroblasts which do not normally express bombesin receptors. The gene for the receptor can be isolated from a genomic library, characterized structurally, and the promoter region used in regulatory studies similar to those planned for the GRP and NM-B gene. Finally, the receptor cDNA coding region will be used as a low-stringency probe to screen genomic and brain cDNA libraries for structurally similar receptor genes.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-00813-28 LNC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Enzymological Aspects of Neural Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                          |                         |            |
|---------|--------------------------|-------------------------|------------|
| PI:     | Robert W. Albers, Ph.D.  | Chief, Enzyme Chemistry | LNC, NINDS |
| Others: | Paul M. Rowe, Ph.D.      | Senior Staff Fellow     | LNC, NINDS |
|         | William T. Link, Ph.D.   | Staff Fellow            | LNC, NINDS |
|         | Charlene P. Osborn, B.S. | Biologist               | LNC, NINDS |

## COOPERATING UNITS (if any)

J.P. Froehlich, Ph.D., M.D., NIA, NIH, Baltimore, MD  
R. Henneberry, Ph.D., NINDS, NIH, Bethesda, MD

## LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

## SECTION

Section on Enzyme Chemistry

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

4.0

## PROFESSIONAL:

3.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is comprised of research into the structure and functioning of ion transport systems. There are currently four active subprojects: 1) studies of the transient state kinetics of phosphorylation and dephosphorylation of the Na,K-ATPase catalytic site, utilizing rapid quenching techniques; 2) a study of the regulation and expression of isoforms of the Na,K-ATPase utilizing site-directed antibodies raised against synthetic peptides as identifying probes; 3) a study of the relation of the glycosylation state of the beta-subunit of the Na,K-ATPase to the expression and function of the sodium pump; and 4) regulation of activity and expression of sodium pumps in neural cell utilizing primary cultures of cerebellar neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01-NS-02723-03 LNC

PERIOD COVERED October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Peptides in the Adult and Developing Vertebrate Nervous Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                              |                     |            |
|------------------------------|---------------------|------------|
| PI: Harold Gainer, Ph.D.     | Chief               | LNC, NINDS |
| Others: Mark Whitnall, Ph.D. | Senior Staff Fellow | LNC, NINDS |
| Kevin Conway, Ph.D.          | Senior Staff Fellow | LNC, NINDS |
| Susan Wray, Ph.D.            | Senior Staff Fellow | LNC, NINDS |
| Sharon Key, B.S.             | Biologist           | LNC, NINDS |
| Carolyn Bondy, M.D.          | Senior Staff Fellow | LNC, NINDS |
| Yoshinobu Hara, Ph.D.        | Visiting Fellow     | LNC, NINDS |

COOPERATING UNITS (if any)

Dr. M. Castel, Hebrew University, Jerusalem, Israel

LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

SECTION

Section on Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

6.0

PROFESSIONAL:

5.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In situ hybridization histochemistry, immunocytochemistry, and  $^3\text{H}$ -thymidine birthday analyses, have provided strong evidence that LHRH neurons in the mouse forebrain derive from progenitor cells in the embryonic olfactory placode. The analysis of aldose reductase mRNA in the rat lens, retina, and kidney showed that expression of this gene is primarily a prenatal event in the retina and lens, possibly related to morphogenesis, and is a postnatal event in kidney related to osmoregulation. The vasopressin containing and vasopressin deficient subpopulations of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus appear to be independently regulated during stress. The co-peptides, dynorphin, CRH, and cholecystokinin, affect vasopressin and oxytocin secretion from the neurohypophysis by different mechanisms. Rat brain organotypic slice cultures have been used to study the effects of estrogen on LHRH gene expression. Estrogen appears to downregulate gene expression of LHRH in a subset of LHRH neurons restricted primarily to the organum vasculosum lamina terminalis (OVLT) region of the brain in female rats.



|   |  |   |
|---|--|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |  | PROJECT NUMBER<br>Z01-NS-02724-03 LNC                                     |
| PERIOD COVERED<br><div style="text-align: center; padding: 5px;">October 1, 1988 through September 30, 1989</div>   |  |   |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)<br><div style="text-align: center; padding: 5px;"><b>Molecular Mechanisms in Neuronal Structure and Function</b></div>  |  |   |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)   |  |   |
| PI: Harold Gainer, Ph.D.<br>Others: Ben Szaro, Ph.D.<br>Shirley House<br>Philip Grant, Ph.D.  | Chief<br>Senior Staff Fellow<br>Biologist<br>Expert Consultant                   | LNC, NINDS<br>LNC, NINDS<br>LNC, NINDS<br>LNC, NINDS                      |
| COOPERATING UNITS (if any)<br><div style="padding: 5px;">L. Charnas, M.D., NICHD; V.M.Y. Lee, Ph.D., University of Pennsylvania</div>   |  |   |
| LAB/BRANCH<br><div style="text-align: center; padding: 5px;"><b>Laboratory of Neurochemistry, DIR, NINDS</b></div>  |  |   |
| SECTION<br><div style="text-align: center; padding: 5px;"><b>Section on Cellular and Developmental Neurobiology</b></div>   |  |   |
| INSTITUTE AND LOCATION<br><div style="text-align: center; padding: 5px;"><b>NINDS, NIH, Bethesda, Maryland 20892</b></div>  |  |   |
| TOTAL MAN-YEARS:<br><div style="text-align: center; padding: 5px;"><b>3.2</b></div>   | PROFESSIONAL:<br><div style="text-align: center; padding: 5px;"><b>2.2</b></div> | OTHER:<br><div style="text-align: center; padding: 5px;"><b>1.0</b></div> |
| CHECK APPROPRIATE BOX(ES)<br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>   |  |   |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)<br><div style="padding: 10px;"> <p>Monoclonal antibodies directed against <u>neurofilament</u> (NF-M) <u>proteins</u> in <u>Xenopus</u>, have been injected into individual blastomeres of <u>Xenopus</u> embryos at the two cell stage. These antibodies remain restricted in the intracellular space so that only cells deriving from the injected blastomere contain the antibody (i.e., about half the cells in the developing embryo contain antibody). Preliminary observations indicate that the axons deriving from neurons containing these antibodies are deficient in <u>axonal outgrowth</u> and <u>branching</u>. A novel neuronal <u>intermediate filament protein</u> (57kDa) has been found and sequenced using <u>molecular cloning</u> techniques. Antibodies to a peptide sequence, deduced from the cDNA sequence, were used in immunocytochemical analyses to show localization of this novel protein specifically to neurons. Studies on rat <u>central and peripheral nervous system</u> neurons <u>in vivo</u> and <u>in vitro</u> have shown that different <u>post-translational modifications</u> (i.e., <u>phosphorylation</u> of specific sites) of neurofilaments are characteristic of specific nerve pathways and neuronal types.</p> </div> |  |   |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02725-03 LNC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Calcium Metabolism and Protein Phosphorylation in Neuronal Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |  |            |
|---------|---------------------------|--|------------|
| PI:     | Harish C. Pant, Ph.D.     | Research Chemist                       | LNC, NINDS |
| Others: | Ayse Dosemeci, Ph.D.      | Visiting Fellow                        | LNC, NINDS |
|         | Alexander Wheaton         | Biological Laboratory Technician       | LNC, NINDS |
|         | Carl Floyd, Ph.D.         | Biologist                              | LNC, NINDS |
|         | Ben Szaro, Ph.D.          | Senior Staff Fellow                    | LNC, NINDS |
|         | James Battey, M.D., Ph.D. | Chief, Molecular Neurosciences Section | LNC, NINDS |
|         | James Way, B.S.           | Biologist                              | LNC, NINDS |

## COOPERATING UNITS (if any)

Dr. P.F. Gallant, LN, NINDS; Dr. Tom Soderling, Vanderbilt University  
 Dr. Jan Metzals, University of Toronto

## LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

## SECTION

Section on Cellular and Developmental Neurobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

4.3

## PROFESSIONAL:

3.2

## OTHER:

1.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Studies have been continued on phosphorylation of neurofilament proteins in mammalian nervous system. A neurofilament-enriched preparation from bovine spinal cord contains endogenous protein kinases that phosphorylate high-, middle-, and low-molecular weight neurofilament subunits (NF-H, NF-M, and NF-L), as well as certain other endogenous and exogenous substrates. A major portion of associated kinases are extracted by treatment of the neurofilament preparation with high salt solution. Assays using specific exogenous substrates, activators and inhibitors for identified kinases revealed regulator dependent and independent kinase activities in the high salt extract. Fractionation of the salt extract on a gel filtration column resolves a regulator-independent kinase activity identified by its ability to phosphorylate preferentially the purified NF-M. We have begun to clone and sequence the squid neurofilament proteins. Using a cDNA probe to a mouse NF protein at low stringency, we screened a cDNA library made from squid optic lobe. We isolated three distinct clones which hybridized with an abundant 5 kilobase RNA on Northern blots of total RNA from squid optic lobe and from stellate ganglia. Two of the three clones overlapped and contained an intermediate filament protein consensus sequence, and additional amino acids highly homologous with amino acids from the 2b portion of the rod domain of the mouse NF protein. By highlighting those portions of the NF protein that are conserved between these two animals, which diverged around 600 million years ago, the sequence of this mRNA will help elucidate functionally important domains of NF proteins.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02753-01 LNC

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of the Genes Encoding Prohormones for Bombesin-like Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |                 |            |
|---------|---------------------------|-----------------|------------|
| PI:     | James Battey, M.D., Ph.D. | Section Chief   | LNC, NINDS |
| Others: | Etsuko Wada, M.D., Ph.D.  | Visiting Fellow | LNC, NINDS |
|         | James Way, B.S.           | Biologist       | LNC, NINDS |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

SECTION

Section on Molecular Neuroscience

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:  
1.8

PROFESSIONAL:  
1.3

OTHER:  
0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects  
☐ (a1) Minors  
☐ (a2) Interviews  
☐ (b) Human tissues  
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand aspects of the structure, function, and regulation of the genes encoding the mammalian counterparts to bombesin-like neuropeptides. Two mammalian bombesin-like peptides have been identified to date, gastrin-releasing peptide (GRP) and neuromedin B (NM-B). Both peptides are found in a subset of central and peripheral neurons, and share a structural motif at their carboxyl-terminal domains, which is necessary and sufficient for binding to high-affinity cell surface bombesin receptors. There are several subprojects being actively pursued: 1) full-length cDNA clones for the rat prepro-Neuromedin B gene, as well as genomic clones, are isolated and structurally characterized, identifying the promoter region and exons; 2) in situ hybridization techniques are being utilized to determine the localization of GRP and NMB mRNA at the cellular level in brain; 3) promoter-reporter gene constructs will be introduced into cultured cell hosts by transfection to map the elements in the promoter region of the rat NM-B and GRP gene responsible for the cell-type specific regulation.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02757-02 LNC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Analyses of Peptide Receptors**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |  |            |
|---------|---------------------------|--|------------|
| PI:     | Kiyoshi Kusano, Ph.D.     | Visiting Scientist                           | LNC, NINDS |
| Others: | Harold Gainer, Ph.D.      | Chief  | LNC, NINDS |
|         | James Battey, M.D., Ph.D. | Chief, Section on<br>Molecular Neurosciences | LNC, NINDS |

## COOPERATING UNITS (if any)

Dr. M. J. Brownstein and Dr. L. Mahan, LCB, NIMH; Dr. I. Tasaki, LCB, NIMH;  
Dr. R. Wenthold, LND, NINDS

## LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

## SECTION

Section on Cellular and Developmental Neurobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects  
☐ (a1) Minors  
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study on the cellular and molecular mechanisms of peptide receptor signal transduction processes has used two approaches. The first approach is to analyze the electrophysiological responses to application of neuropeptides in selected peptide receptor-bearing cells: Swiss 3T3 cells for bombesin receptors and AR42J cells for cholecystokinin (CCK) receptors. The second approach is to study the peptide receptor signal transduction process in detail using the Xenopus oocyte surrogate system, after injection of mRNA's extracted from the above cells. Whole cell voltage-clamp and patch-clamp techniques are applied to those cultured cells. The Swiss 3T3 cells do not have profound voltage sensitive K-channels, but some cells possess a transient type of Ca-current (I Ca(t)) channels. Application of bombesin to 3T3 cells induces a K-conductance increase by activating a Ca-mediated K-current (I K[Ca]) flow. AR42J cells possess voltage-sensitive I Ca(t), I K(Ca), I Cl(Ca) and also I Na in a small number of cells. Application of CCK to AR42J cells induces a conductance increase to Cl-, whereas Na+ and K+ do not show significant effects. In both cell lines ligand binding with receptors triggers a rise in intracellular Ca2+ concentration by release from intracellular sources. The two-electrode voltage clamp technique has been applied to assay the functionally transcribed CCK and bombesin receptors in the Xenopus oocytes, after injection of their respective mRNAs. Size fractionated mRNAs obtained by the sucrose gradient technique are further assayed in order to clone and sequence these receptor cDNAs and genes.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02774-01 LNC

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Molecular Cloning of Bombesin Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |                    |            |
|---------|---------------------------|--------------------|------------|
| PI:     | James Battey, M.D., Ph.D. | Section Chief      | LNC, NINDS |
| Others: | Kiyoshi Kusano, Ph.D.     | Visiting Scientist | LNC, NINDS |
|         | Etsuko Wada, M.D., Ph.D.  | Visiting Fellow    | LNC, NINDS |
|         | Yoshinobu Hara, Ph.D.     | Visiting Associate | LNC, NINDS |
|         | James Way, B.S.           | Biologist          | LNC, NINDS |

## COOPERATING UNITS (if any)

Richard Feldman, Ph.D., and Susan Stuart, Ph.D.

Triton Biosciences, 1501 Harbor Way Parkway, Alameda, CA 94501

## LAB/BRANCH

Laboratory of Neurochemistry, DIR, NINDS

## SECTION

Section on Molecular Neuroscience

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.8

## PROFESSIONAL:

1.3

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects  
☐ (a1) Minors  
☐ (a2) Interviews

☐ (b) Human tissues☒ (c) Neither

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this newly initiated project is to use molecular genetic techniques to study the structure and function of bombesin receptor(s). We plan to accomplish this by first obtaining a full-length cDNA clone for the murine bombesin receptor. The cDNA will be sequenced to determine the predicted amino acid sequence and general structural features of the receptor. Specific antisera will be generated against synthetic peptides to allow further characterization of the receptor protein. This cDNA clone can then be used in site-specific mutagenesis studies where the effects of structural perturbations can be examined after expression of the mutant receptors in Xenopus oocytes and DNA-mediated transfection into murine fibroblasts which do not normally express the receptor. Genomic clones will be isolated to examine the gene structure and promoter region. The cDNA coding domain will be used as a low-stringency probe to screen brain cDNA libraries for other distinct but structurally related bombesin receptor cDNA clones.





# ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Neuropathology and Neuroanatomical Sciences  
Basic Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT  
October 1, 1988 through September 30, 1989  
Laboratory of Neuropathology and Neuroanatomical Sciences  
Basic Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

Igor Klatzo, M.D. Chief

During the past year, the Laboratory of Neuropathology and Neuroanatomical Sciences has embarked upon a study of pathophysiological areas of cerebral ischemia, which in spite of their exceptional clinical relevance and importance, have been until recently neglected and little explored. These areas concern the problems of chronic ischemic neuronal injury developing after a single or repeated ischemic insults. It can be assumed that such chronic neuronal changes may constitute a major pathophysiological process especially in brains of aging patients with a certain degree of cerebrovascular insufficiency.

The delayed neuronal death, first described in this laboratory (Ito et al., 1975), led to a concept of "maturation phenomenon" i.e., ripening of ischemic injury, which can be elicited in various parameters of ischemic pathophysiology. According to our recent studies, the delayed neuronal injury extends beyond the well described selective destruction of pyramidal cells in CA1 sector of hippocampus and it may be recognized also in various neuronal areas of the brain, sometimes outside of ischemic territory.

Evaluating various changes during postischemic periods we have found an increasing number of clues pointing to neuroexcitatory mechanisms as responsible for the development of transneuronal lesions which contribute to the ultimate outcome of ischemic injury.

An assumption that neuroexcitatory mechanisms play an important part in ischemic pathophysiology was strongly supported by the discovery of transneuronal secondary foci of injury developing after some delay following cryogenic cold lesions. Thus our work becomes currently focused on the theory that neuroexcitation may be an important component of brain injury of different etiology, such as ischemia, trauma, hypoglycemia, convulsive disorders, etc., and therefore an effective suppression of neuroexcitation could be beneficial in the treatment of various mentioned conditions.

The Section of Cerebrovascular Pathology has started investigations concerning the role of neuroexcitation in pathomechanism of various brain injuries in several new projects with reference to: a) effects of repeated ischemic insults on the pattern of calcium uptake, cerebrovascular permeability and morphological changes, b) effects of cryogenic cortical lesions on the development of transneuronal distant lesions, and c) effects of temporary global ischemia in rats involving the study of acute and long-term, chronic changes.

Studies on repeated ischemic insults revealed different patterns of ischemic injury than those observed following single, temporary arterial occlusions.

Concerning abnormal uptake and accumulations of calcium the main differences between single and repeated occlusions referred to topography and chronology of changes. Whereas in the single, bilateral 5 min common carotid occlusions in gerbils the abnormal accumulations of calcium were confined to the hippocampus and occasionally to the lateral portions of the caudate, in gerbils with three repeated 2 and 5 min occlusions, spaced at 1 hr intervals, the most pronounced feature was intense calcium uptake in the thalamus, the middle geniculate bodies and after 4 days in the substantia nigra.

Electron microscopic observations on calcium using an oxalate-pyranthionate precipitation method showed abnormal accumulations of calcium precipitates conspicuously in the cytosol of dendrites in the affected areas. The mitochondrial uptake of calcium was observed either in presumably pyramidal neuronal elements of CA1 sector undergoing acute destruction, or after long periods of post-ischemic survival, compartmented in the center of otherwise well preserved mitochondria in hippocampal neuronal elements of other than pyramidal cells.

The observations on the behavior of the blood-brain barrier (BBB) using horseradish peroxidase (HRP) or by immunocytochemical demonstration of serum albumin in brain sections revealed changes in permeability of the BBB which paralleled in localization and time of appearance the changes in abnormal accumulation of calcium. Using immunocytochemical method the extravasated albumin was frequently observed localized in granular form in the cytoplasm of neurons in the affected areas.

In preliminary assays, autoradiographic evaluation of deoxyglucose utilization revealed in gerbils subjected to three 5 min occlusions and sacrificed 3 hrs after the last insult, a striking increase of glucose utilization in thalamic nuclei, which disappeared in animals sacrificed at later time intervals.

A serendipitous observation indicating that a trauma to cerebral cortex alone may lead to calcium accumulation and neuronal injury in a distant subcortical brain region prompted us to study mechanisms involved in this phenomenon.

Our studies in gerbils and rats revealed that application of a cryogenic probe to various regions of the skull can produce distinct foci of injury in various subcortical brain regions, dependent on location of cryogenic cortical injury. Thus injuries of somatomotor cortex were associated often with ipsilateral thalamic foci, injury of entorhinal cortex in gerbils frequently resulted in bilateral involvement of the hippocampus. These distant foci were recognizable after 48 hrs showing similar features described above in repetitive ischemia: there was a pronounced accumulation of calcium which ultrastructurally showed the same pattern of distribution, predominantly in the dendrites; there were similar changes in permeability of the BBB, with extravasated serum albumin being seen mostly in the cytoplasm of neuronal elements. Morphologically the secondary foci revealed pictures of neuronal injury, with predominance of dark neurons with darkly stained distorted processes. Sometimes there was also noticeable vacuolization of the neuropil.

The observations from repetitive ischemic insults and cryogenic cortical lesions with regard to delayed appearance of neuronal changes, characteristic pattern of predominantly dendritic calcium uptake, BBB permeability changes, as well as evidence of increased metabolic rate, preceding neuronal injury, resemble closely changes observed in convulsive seizures associated with strong neuroexcitation and

thus, they strongly suggest a possibility of neuroexcitatory mechanisms playing the major role in post-ischemic and post-traumatic development of the lesions.

To pursue further elucidation of mechanisms involved in delayed and chronic neuronal injury we introduced a modified model of temporary global ischemia in rats, originally described by Korpachev (1982). This model reproduces closely conditions encountered in cardiac arrest and therefore is of considerable clinical significance. Otherwise, it appears to be the only available experimental model suitable for study of chronic, long-term sequelae of global ischemia.

By using a device of a bent lumbar puncture needle which introduced below the heart allows compression of all major vessels caught between the needle and finger pressure applied from outside, a global ischemia of 10 minute duration is produced (continuously monitoring CBF with laser Doppler, BP, EEG and EKG). Although the mortality of the procedure is 20-30%, the resuscitated animals show excellent recovery and can be used for long-term observations.

Our preliminary observations reveal ischemic injury evident from the first day after resuscitation involving neuronal changes and abnormal accumulation of calcium, initially observed in the cerebral cortex, striatum and hippocampus. After 7 days the changes appear in the thalamus, substantia nigra and other neuronal structures. Brains of rats sacrificed after 4 weeks revealed conspicuous presence of darkly stained neurons with increased stainability of their processes, resembling those described as a chronic, ischemic degeneration. Our current studies on this model include a profile assessment of metabolic, protein synthesis, calcium uptake, BBB changes and electrophysiological recordings to elucidate to what extent neuroexcitatory mechanisms can play a part also in this model.

In summary, similar features observed in the described three projects (on repeated ischemia, cryogenic cortical injury and global ischemia) seem to suggest a possible involvement of a common factor, such as neuroexcitation which may play a significant role in the final outcome of postischemic and posttraumatic lesions. Our current effort is directed towards solid establishment of neuroexcitatory nature of some of the changes observed during postischemic and posttraumatic periods by applying direct electrophysiologic recording, biochemical determinations of excitatory amino acids, as well as by assays on energy metabolism and protein synthesis changes. After analyzing our data, attempts will be made to influence the course of postischemic and posttraumatic lesions by testing various agents which could interfere and reduce the element of neuroexcitation.

The efforts of Dr. Nowak continue to be focused on evaluating changes in gene expression which occur in brain after ischemia and other insults. In the past year the use of in situ hybridization has allowed the determination of the distribution and time course of postischemic induction of the mRNA encoding the major stress/heat shock protein, hsp70, and these techniques have also been applied to the study of the proto-oncogene, c-fos, and the opioid peptide precursor, prodynorphin. From these studies there has emerged a consistent pattern of apparently activity-related changes in neuronal gene expression after ischemia.

Hsp70 mRNA is prominently expressed throughout hippocampal circuitry within several hours following an ischemic insult. This expression is transient in dentate granule cells and CA3 neurons which eventually accumulate immunoreactive hsp70 protein, but hybridization persists in CA1 neurons which fail to express the protein, essentially until the death of these neurons. The



anticonvulsant MK-801 considerably attenuates the early induction of hsp70 mRNA but has no apparent effect on the later expression in CA1. This pharmacological result supports the suggestion that hsp70 induction is a consequence of increased neuronal activity following ischemia, and the failure to alter hsp70 induction in CA1 is consistent with the still controversial observations of this laboratory and others that single doses of MK-801 also fail to prevent postischemic CA1 neuron loss.

The proto-oncogene, *c-fos*, is very transiently induced during 15-60 min after ischemia. Although there may be slight mRNA induction in CA3 and CA1 the increase is most evident in dentate granule cells, and *c-fos* hybridization thus appears to strongly label only a subset of the cell populations which later show pronounced hsp70 induction. Interestingly, there is a significant 50% decrease in hybridization of prodynorphin sequences in dentate granule cells after ischemia, consistent with previous observations of reduced dynorphin peptide levels in hippocampus. Reciprocal *c-fos* induction and prodynorphin down-regulation have also been observed in seizure models, indicating that the present results are a consequence of excitatory activity in hippocampus after ischemia.

Future studies with MK-801 and other compounds may be expected to confirm the activity-dependent nature of the changes in neuronal gene expression after ischemia. More extensive mapping with the hsp70 probe may identify hybridization in circuitry outside hippocampus which may correlate with the persistent phase of hsp70 induction in CA1, and therefore be identified as candidates for mediating proposed excitotoxic mechanisms of cell death in this region. In general, the longer time course of hsp70 induction suggests that it could be more practical than the extremely transient *c-fos* expression for mapping circuitry which is activated following ischemia and other stresses, although the precise signal for hsp70 induction remains to be determined. This may be applicable to the study of circuitry involved in the increased vulnerability of thalamus and other regions after repeated ischemic insults. Finally, inhibition of hsp70 induction, particularly the lasting expression in CA1, should provide a useful marker for screening drugs of potential benefit in preventing postischemic neuronal loss.

In other immunocytochemical experiments it has been confirmed that hsp70 induction in brain following a classical hyperthermic stress is largely glial, with the one identified exception being cerebellar granule cell neurons. However, primary cultures of neonatal rat cerebellar granule cells fail to show significant hsp70 induction while astrocyte cultures exhibit a robust heat shock response. The molecular basis for selective hsp70 induction in glial elements *in vivo*, and the mechanisms responsible for the differential granule cell response *in vivo* and *in vitro*, remain problems of fundamental importance to be approached in future studies.

The continuous goals of the Section on Neurocytobiology have been: I. to develop and utilize new model systems for the investigation of basic mechanisms operative on the level of normal and pathologically altered blood-brain barrier (BBB) and cerebral blood flow (CBF); II. to study the metabolic processes occurring in cerebral ischemia and ischemic edema, their prevention and therapy; III. to evaluate the influence of genetic and immunological factors on the generation of experimental autoimmune diseases (including ischemia) involving central nervous system.

I. During the last year, the separately cultured endothelial cells derived from dissociated cerebral microvessels of both rats and SJL mice have been very useful models for the continuous studies of cerebrovascular function related to the BBB

and CBF. Moreover, we have successfully developed and established cultures of human endothelium derived from brain microvessels.

Three different aspects of cerebral microvascular endothelial properties related to the function of BBB and the regulation of CBF have been further investigated in the in vitro models using pure endothelial cell cultures: a) the role of arachidonic acid alone or with peroxidation on the cellular permeability, b) detection and characterization of endothelial receptors linked to adenylate cyclase (AC), and c) interaction between cerebral capillary endothelium and immune cells.

a) The relationship of free arachidonic acid (AA) to cellular permeability, lipid peroxidation and physical state "fluidity" of the membrane was investigated in cultured endothelial cells (EC) dissociated from cerebral microvessels of rats. The results demonstrate that AA can induce a reversible alteration of endothelial permeability to trypan blue albumin (TBA). Exposure of EC to AA increases membrane "fluidity" as measured by fluorescence anisotropy using 1,6-diphenyl-1,3,5 hexatriene as a fluorescent probe. The AA modification of EC membrane "fluidity" is not associated with changes in EC permeability. Addition of AA and  $H_2O_2$  to the incubation medium of EC leads to persistent alteration of EC permeability which can be prevented by catalase treatment. Both AA and  $H_2O_2$  induce a greater formation of malondialdehyde, the product of lipid peroxidation, than AA alone. These findings strongly suggest that a release of AA either from the capillary or cellular membrane of the brain under a pathological condition may alone or through a peroxidative process alter the function of blood-brain barrier.

b) Neurotransmitters and/or vasoactive substances are implicated in regulation of cerebromicrovascular function. The inaccessibility of cerebromicrovessels hindered investigation of regulatory mechanisms. So far, cultured cellular constituents of microvessels separated from brain of various animals but not man were shown to be useful for elucidating some of the mechanisms.

This investigation represents a part of a long term study that has been aimed to shed light on endothelial mechanisms involved in regulating the function of cerebromicrovascular compartment. However, at the present time, the investigation of human cerebromicrovascular endothelium has been designed to acquire the most information with limited resources. Thus, responsiveness of endothelial adenylate cyclase (AC) system to various vasoactive substances was evaluated in homogenates of cultured endothelial cells (EC) derived from three fractions of human brain microvessels to various vasoactive substances. All cell cultures stain positively with human Factor VIII-related antigen and only occasionally a positive GFAP cell was found in some cultures. Endothelial AC system was responsive to adrenergic, dopaminergic, serotonergic,  $PGD_2$  and  $PGE_2$  substances (all at  $10^{-5}M$ ) irrespective of its origin. The sensitivity of AC to adrenergic agents was greater in the capillary endothelium (6-fold over BA) than in arteriolar EC (2-fold over BA). The AC responsiveness to dopamine, 5-HT,  $PGD_2$  and  $PGE_2$  was greatest in EC derived from small arterioles and venules. Prostaglandins F-type ( $10^{-5}M$ ) stimulated AC activity of the endothelium derived from arterioles and venules while bradykinin ( $10^{-5}M$ ) enhanced the activity of capillary and small arteriolar ECs. The enhancement of AC activity to adrenergic substances could be inhibited by butoxamine ( $\beta_2$ -blocker) and prazosin ( $\alpha_1$ -blocker) indicating the presence of  $\beta_2$ - and  $\alpha_1$ -adrenergic receptors linked to AC in all EC fractions. The presence of  $D_1$ - but not  $D_2$ -receptors was indicated by inhibition of dopamine AC response with  $D_1$  but not  $D_2$ -antagonists [SCH-23390, R(+) and sulpiride, S(-), respectively]. The presence of 5-HT $_1$ -receptors linked to AC was suggested by mianserin (5-HT $_1$  > 5-HT $_2$  antagonist)

inhibition of 5-HT effect on AC activity and ineffectiveness of  $\alpha$ -methylserotonin (5-HT<sub>2</sub> agonist). The response of adenylate cyclase to acetylcholine, carbachol and histamine was seen in all examined homogenates of endothelium (4th-11th passage) derived from large microvessels. Pirenzepine (M<sub>1</sub>-muscarinic inhibitor) blocked the acetylcholine and carbachol stimulated formation of cAMP while methoctramine (M<sub>2</sub> muscarinic inhibitor) had no effect on AC of endothelium. The response of endothelial AC activity to histamine was inhibited (30-100%) by cimetidine indicating the AC linkage to H<sub>2</sub>-receptors. These findings suggest that the endothelial AC responsiveness to vasoactive substances is related to the microvascular origin of EC. This study represents a first demonstration of cultured human cerebral endothelium derived from microvessels of different sizes showing not only the same but different receptors linked to AC activity.

c) The continuous investigation of cerebrovascular endothelial role in the development of immune reactivity in the central nervous system (CNS) has been eminently expanded since Dr. Richard McCarron joined our Section. The possibilities of investigating various aspects of this project were also aided by the development and establishment of EC cultures derived from microvessels of SJL mice susceptible to EAE.

The presence of the BBB in addition to the absence of conventional lymphatic drainage, has led to a traditional view of the brain as an immunologically privileged site. There is a growing body of evidence which indicate that the CNS is not so "privileged" and that EC which compose the BBB play active roles in a number of interactions involving a variety of cells, such as white blood cells, PMS leukocytes and astrocytes. There is also recent evidence that alterations in BBB permeability seen during the CNS autoimmune disease experimental allergic encephalomyelitis (EAE), correlated with changes in the expression of EC surface antigens.

Previously, we demonstrated that cerebral EC can be induced by interferon-gamma to synthesize and express Ia antigen on their surfaces. It was also shown that these positive EC can function as antigen-presenting cells to MBP-reactive T cells which can adoptively transfer EAE. Since this interaction between EC and lymphocytes may result in the elaboration of factors which affect BBB integrity and permit entry of lymphocytes and other immune cells (plasma cells, macrophages) into the brain, the regulation of Ia antigen expression by EC may be an important factor in the pathogenesis of neurological diseases such as EAE.

In the present study attempts have been made to understand the mechanism of IFN $\gamma$  induction of Ia antigen expression on EC and to modulate the expression with a variety of factors. Experiments are conducted which: 1) characterize adrenergic receptors on brain EC; 2) measure formation of cAMP; and 3) quantitate expression of Ia antigen.

#### Interferon-induced Ia expression

Murine cerebral microvascular EC culture do not constitutively express Ia antigens. IFN $\gamma$  induced Ia antigen expression on EC in a time- and dose-dependent manner. The optimal conditions for induction were observed after three days culture in the presence of 100 U/ml IFN $\gamma$ . EC cultures grown in the presence of isoproterenol ( $\beta_2/\beta_1$ -adrenergic agonist and activator of cAMP dependent protein kinase A) and/or forskolin (activator of catalytic unit of AC) exhibited significantly lower levels of Ia antigen expression. Forskolin also augmented the isoproterenol inhibition of IFN $\gamma$ -induced Ia expression. Propranolol ( $\beta_2/\beta_1$ -adrenergic antagonist)



and to a lesser extent yohimbine, ( $\alpha_2$ -adrenergic antagonist) were able to partially block the ability of isoproterenol to inhibit Ia induction by IFN. EC cultures treated with IFN $\gamma$  in the presence of PMA (activator of protein kinase C) demonstrated higher levels of Ia expression than were seen in cultures containing only IFN $\gamma$ . In addition, PMA reduced the inhibitory effect of forskolin on IFN $\gamma$  induction of Ia.

#### Correlation between cAMP production and Ia induction

Isoproterenol treatment resulted in the increased formation of cAMP and an inhibition of the IFN $\gamma$ -induced expression of Ia antigen. Propranolol partially blocked the cAMP production induced by isoproterenol and reduced the isoproterenol inhibitory effect on IFN $\gamma$ -induction Ia antigen. On the other hand, yohimbine increased the isoproterenol-induced stimulation of cAMP and decreased the inhibitory effect of isoproterenol on IFN $\gamma$ -induced Ia expression. Also, PMA augmented (synergistically) the forskolin enhancement of cAMP production and reduced the inhibitory effect of forskolin on Ia induction by IFN $\gamma$ . Clonidine,  $\alpha_2$ -adrenergic agonist which did not affect forskolin but reduced isoproterenol stimulation of cAMP formation, had no effect on the modulation of IFN $\gamma$ -induced Ia expression seen by these substances.

These results support the concept that agonists and antagonists of adrenergic receptors can modulate the induction of Ia expression. The mechanism involves the interaction between the two signal transduction pathways leading to the activation of protein kinase A and cAMP and the activation of protein kinase C.

Dr. McCarron's independent investigations involved the comparison between TNF and interleukin-1 (IL-1) effects on EC and those on astrocytes. TNF inhibited Ia induction by IFN in a dose-dependent manner. The degree of inhibition by TNF was related to the IFN concentration: 200 U/ml TNF inhibited Ia expression induced by 20 U/ml IFN by 80% and Ia induced by 100 U/ml IFN by 45%. FACS analysis revealed the induction of Ia antigen on 30-40% of EC after three days culture with IFN, TNF significantly reduced the percent of EC expressing Ia antigens. Identical treatment of SJL astrocytes showed TNF augmented Ia expression induced by IFN.

IL-1 also inhibited Ia induction by IFN in a manner similar to that observed with TNF. The percent reduction of Ia positive EC by IL-1 (2.0 U/ml) was 30% and 50% during incubations with 100 and 20 U/ml IFN, respectively. When combined at suboptimal concentrations IL-1 and TNF synergistically inhibited Ia expression induced by IFN. These results demonstrate that TNF acts on EC and astrocytes in a disparate manner and indicate that TNF and IL-1 can synergistically down regulate immune responses involving cerebral EC.

These studies involved the interaction between brain EC and myelin basic protein (MBP)-reactive T lymphocytes which adoptively transfer chronic relapsing EAE in naive syngeneic mice. The capacity of cerebral capillary EC to serve as a target for these T lymphocytes [which can function as cytotoxic T lymphocytes (CTL)] was examined.

The kinetics of the CTL mediated lysis of EC targets follows the classical fashion (i.e., direct relationship between percent specific lysis and effector:target ratio). The CTL response was class II MHC-restricted and required the expression of Ia antigen on the target (EC) surface. Anti-Ia antibody pretreatment of targets blocks the lytic destruction; also Ia-negative cells were not affected, moreover, only syngeneic combination of EC targets and CTL's resulted in lysis. The CTL response

was also antigen-specific; EC targets incubated in the absence of antigen or pulsed with irrelevant antigen were unaffected.

In addition to functioning as targets, EC also interacted with lymphocytes during the generation of proliferative responses. Although EC could function as antigen-presenting cells for purified T cell populations, the addition of EC to whole lymph node cell populations resulted in the inhibition of antigen-specific proliferative responses. This inhibition could be reproduced by adding EC culture supernatants and could be blocked by the inclusion of indomethacin in EC cultures. Analysis of EC supernatants indicated the presence of high (inhibitory) concentrations of various prostaglandin species (PGI as well as PGE). These data indicate that the lymphocytes interaction may provide potential mechanisms for the pathological alteration of BBB, resulting in vascular egress of cells which might lead to neuropathological lesions observed in autoimmune diseases such as multiple sclerosis.

II. The continuous studies of cerebral ischemia, its pathophysiology, prevention and therapy have been focused on a) the elucidation of the effects of ischemia on metabolic pathway of 5-HT, and b) the relationship between ischemic changes in the cerebral content of energy and noradrenergic metabolites in adult and young animals.

a) The postischemic persistent disturbance of 5-HT in the brain is associated with an increased content of free tryptophan. To further evaluate the mechanism responsible for these observations we investigated the effect of ischemia on synaptosomal uptake of tryptophan and its conversion to 5-HT and 5-HIAA.

Cerebral ischemia was induced in gerbils by bilateral carotid artery occlusion for 15 min with and without 1 hr release. Tryptophan, 5-HT and 5-HIAA content were simultaneously measured (HPLC) in cerebrocortical synaptosomes prior to and after tryptophan addition 5 $\mu$ M/mg protein according to Wolf and Kahn method (J Neurochem 46:61, 1986).

Ischemia alone had no effect on the intrasynaptosomal uptake of tryptophan and its conversion to 5-HT and 5-HIAA. However, the 5-HT and 5-HIAA remained significantly decreased (33%) as compared to controls (100%). In postischemia, the tryptophan uptake was not significantly different from that of controls. The conversion of tryptophan to 5-HT and 5-HIAA was 40% lower than the de novo synthesized indolamines from the exogenous tryptophan. These findings are consistent with our previous observations of postischemic persistent dysfunction of cerebrocortical synaptosomes.

b) Changes in the brain content of biogenic amines belong to one of the main factors implicated in the pathogenesis of ischemic CNS damage. We have recently demonstrated a lack of correlation between the cortical ischemic changes of energy related metabolites and noradrenergic metabolites in the adult or the young gerbil brains. The disturbance of the NE metabolic pathway occurred at the time of recovery of energy reserves in adults while in the young animals the absence of a significant effect of ischemic insult on the noradrenergic system contrary to that observed on serotonergic metabolites.

The study represents a continuous effort to assess the relationship between the ischemic cerebral monoamine changes and the lesser susceptibility of young than adult animals to ischemic insult.

A significant decrease in DA, NE, DOPAC and HVA was only seen after 15 min ischemia in adult but not in young striatum. Tryptophan and HIAA level was 2-3 fold higher in adults after 5 min ischemia and 1 reflow. Similar changes were found in both groups of animals after 15 min ischemia and reflow. At the same time a persistent reduction of DA and NE was observed in adults but that of DA only in young animals.

These findings are consistent with our previous suggestion that the relative resistance of young animals may be partly related to the function of neurotransmitters in the CNS.

\* III. Dr. McCarron has also initiated himself in vivo studies of passive transfer of myelin basic protein (MBP)-reactive T cells to variously susceptible mice. Experimental allergic encephalomyelitis (EAE) can be induced in naive mice by the passive transfer (MBP)-reactive T cells. Specific encephalitogenic MBP epitopes, which differ according to class II phenotype, are required for the induction of EAE in susceptible animals. MBP residues 89-101 are encephalitogenic in SJL mice (H-2<sup>s</sup>) while residues 1-9 are encephalitogenic in PL mice (H-2<sup>d</sup>). Both of these determinants are encephalitogenic in SJLxPL (F1) mice. Utilizing purified F1 T cells from mice immunized with either MPB fraction 1-37 or 89-169 and cultured in the presence of either PL (H-2<sup>d</sup>) or SJL (H-2<sup>s</sup>) APC, respectively, EAE was induced in F1 mice by passive transfer. It was observed that during the course of chronic relapsing EAE, new T cell antigen specificities and class II restriction requirements were generated. This indicates the possibility that antigens distinct from those required to induce EAE may be involved in the onset of subsequent relapses during the course of disease.



|  |              |   |
|--|--------------|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |              | <b>PROJECT NUMBER</b><br><br>Z01 NS 02324-12 LNNS |
| <b>PERIOD COVERED</b><br>October 1, 1988 to September 30, 1989   |              |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Blood-Brain Barrier: In Vitro Model for the Study of Cerebrovascular Endothelial Permeability  |              |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)   |              |   |
| PI:  | M. Spatz     | Section Chief<br>LNNS, NINDS                      |
| Other:   | A. Villacara | Visiting Fellow<br>LNNS, NINDS                    |
| <b>COOPERATING UNITS</b> (if any)  |              |   |
| <b>LAB/BRANCH</b><br>Laboratory of Neuropathology and Neuroanatomical Sciences   |              |   |
| <b>SECTION</b><br>Section of Neurocytobiology  |              |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892  |              |   |
| TOTAL MAN-YEARS:   | 0.8          | PROFESSIONAL: 0.6<br>OTHER: 0.2                   |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>   |              |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  |              |   |
| <p>Recently, we demonstrated a reversible change in cerebromicrovascular permeability and the presence of increased membrane "fluidity" of the endothelium exposed to free arachidonic acid (AA). This investigation represents a continuation of the studies concerned with elucidation of mechanisms responsible for alteration of cellular endothelial permeability under pathological conditions. It focuses on the effect of AA alone or in the presence of H<sub>2</sub>O<sub>2</sub> on endothelial lipid peroxidation <u>in vitro</u> and its relationship to changes in cellular permeability.</p> <p>The results demonstrate that AA can induce a reversible alteration of endothelial permeability to trypan blue albumin (TBA) while cells incubated with AA and H<sub>2</sub>O<sub>2</sub> showed an irreversible increase in TBA permeability.</p> <p>Both AA and H<sub>2</sub>O<sub>2</sub> induce a greater formation of malondialdehyde, the product of lipid peroxidation, than AA alone. These findings strongly suggest that a release of AA either from capillary or cellular membrane of the brain under a pathological condition may alone or through a peroxidative process alter the function of blood-brain barrier.</p> |              |   |
| 10 - LNNS/DIR  |              |   |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02357-11 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia and Monoamines

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Spatz Section Chief LNNS, NINDS

Others: K. Kumami Visiting Fellow LNNS, NINDS  
CJ Chang Visiting Fellow LNNS, NINDS

## COOPERATING UNITS (if any)

Institute of Biochemistry, Faculty of Medicine, Belgrade, Yugoslavia, (Bogomir.B. Mrsulja)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Neurocytobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.8 PROFESSIONAL: 0.2 OTHER: 0.6

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Changes in the brain content of biogenic amines belong to one of the main factors implicated in the pathogenesis of ischemic CNS damage. We have recently demonstrated a lack of correlation between the cortical ischemic changes of energy related metabolites and noradrenergic metabolites in the adult or the young gerbil brains. The disturbance of the NE metabolic pathway occurred at the time of recovery of energy reserves in adults while in the young animals, the absence of a significant effect of ischemic insult on the noradrenergic system contrary to that observed on serotonergic metabolites (Spatz et al. 1985) is of special interest.

This study represents a continuous effort to assess the relationship between the ischemic cerebral monoamine changes and the lesser susceptibility of young than adult animals to ischemic insult.

A significant decrease in DA, NE, DOPAC and HVA was only seen after 15 min ischemia in adult but not in young striatum. Tryptophan and HIAA level was 2-3 fold higher in adults after 5 min ischemia and 1 reflow. Similar changes were found in both groups of animals after 15 min ischemia and reflow. At the same time a persistence of DA and NE were observed in adults but that of DA only in young animals.

These findings are consistent with our previous suggestion that the relative resistance of young animals may be partly related to the function of neurotransmitters in the CNS.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02548-08 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Electrical Impedance in Cerebral Ischemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |             |                    |             |
|---------|-------------|--------------------|-------------|
| PI:     | H.G. Wagner | Scientist Emeritus | LNNS, NINDS |
| Others: | S. Xu       | Visiting Fellow    | LNNS, NINDS |
|         | I. Klatzo   | Chief              | LNNS, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.2

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Further study was made of cerebral electrical impedance (CEI) during the phase of hypoperfusion. In the gerbil no substantial decrease in the CEI was found during this phase after a single 5 minute bilateral carotid occlusion. This was in spite of noticeable swelling of the brain and probable compression of the capillaries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02623-06 LNNS

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia and Edema: Tryptophan Uptake and Metabolism\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |            |                 |             |
|---------|------------|-----------------|-------------|
| PI:     | C.J. Chang | Visiting Fellow | LNNS, NINDS |
| Others: | K. Kumami  | Visiting Fellow | LNNS, NINDS |
|         | M. Spatz   | Section Chief   | LNNS, NINDS |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

|                  |     |               |     |        |     |
|------------------|-----|---------------|-----|--------|-----|
| TOTAL MAN-YEARS: | 0.9 | PROFESSIONAL: | 0.7 | OTHER: | 0.2 |
|------------------|-----|---------------|-----|--------|-----|

CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The postischemic persistent disturbance of 5-HT in the brain is associated with an increased content of free tryptophan. To further evaluate the mechanism responsible for these observations we investigated the effect of ischemia on synaptosomal uptake of tryptophan and its conversion to 5-HT and 5-HIAA.

Cerebral ischemia was induced in gerbils by bilateral carotid artery occlusion for 15 min with and without 1 hr release. Tryptophan, 5-HT and 5-HIAA content were simultaneously measured (HPLC) in cerebrocortical synaptosomes prior to and after tryptophan addition 5µM/mg protein according to Wolf and Kahn method (J. Neurochem. 46:61,1986).

Ischemia alone had no effect on the intrasynaptosomal uptake of tryptophan and its conversion to 5-HT and 5-HIAA. However, the 5-HT and 5-HIAA remained significantly decreased (33%) as compared to controls (100%). In postischemia, the tryptophan uptake was not significantly different from that of controls. The conversion of tryptophan to 5-HT and 5-HIAA was 40% lower than the *de novo* synthesized indolamines from the exogenous tryptophan. These findings are consistent with our previous observations of postischemic persistent dysfunction of cerebrocortical synaptosomes.

\*Formerly: "Ischemic Brain Edema: 5-HT Receptors."

|   |  |   |
|---|--|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |  | <b>PROJECT NUMBER</b><br><br>Z01 NS 02689-05 LNNS |
| <b>PERIOD COVERED</b><br>October 1, 1988 to September 30, 1989  |  |   |
| <b>TITLE OF PROJECT</b> <i>(80 characters or less. Title must fit on one line between the borders.)</i><br>Regulation of Carbohydrate Metabolism in Cerebromicrovascular Cultures   |  |   |
| <b>PRINCIPAL INVESTIGATOR</b> <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i><br>PI:                      M. Spatz                      Section Chief                      LNNS, NINDS  |  |   |
| <b>COOPERATING UNITS</b> <i>(if any)</i><br>David Lust, Case Western Reserve University, Cleveland, Ohio<br>B.B. Mrsulja, CVD Research Group, Institute of Biochemistry, Belgrade, Yugoslavia   |  |   |
| <b>LAB/BRANCH</b><br>Laboratory of Neuropathology and Neuroanatomical Sciences  |  |   |
| <b>SECTION</b><br>Section of Neurocytobiology   |  |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892   |  |   |
| <b>TOTAL MAN-YEARS:</b><br><div style="display: flex; justify-content: space-between; width: 100%;"> <span>0.6</span> <span>PROFESSIONAL: 0.3</span> <span>OTHER: 0.3</span> </div>   |  |   |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>  |  |   |
| <b>SUMMARY OF WORK</b> <i>(Use standard unreduced type. Do not exceed the space provided.)</i><br><br><p>Studies <u>in vitro</u> related to neurogenic regulation of cerebromicrovascular function showed an involvement of <math>\alpha 2</math>-adrenergic system in controlling NE inducible glycogenolysis in separately cultured cerebromicrovascular cellular elements. The present investigation focused on the responsiveness of phosphorylase a and b to adrenergic agonist and antagonist in order to elucidate the possible mechanisms responsible for NE inducible glycogenolysis.</p> <p>The study is still in progress and no sufficient data were generated for a proper evaluation.</p> |  |   |



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02718-04 LNNS

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Cerebral Electrical Activity Associated with Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.G. Wagner Scientist Emeritus LNNS, NINDS

Other: S. Xu Visiting Fellow LNNS, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

|                  |     |               |     |        |     |
|------------------|-----|---------------|-----|--------|-----|
| TOTAL MAN-YEARS: | 0.9 | PROFESSIONAL: | 0.8 | OTHER: | 0.1 |
|------------------|-----|---------------|-----|--------|-----|

CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An effort has been made to detect and analyze functionally resolvable single units in gerbil brain regions affected by repeated bilateral carotid occlusions or cold lesions. In normal uninjured brain, spontaneous activity has been relatively easy to recover in places like the hippocampus, less frequently found are usable single units in the cortex. Injury discharges are common and indicate that many neurons are present around the electrode but are "silent" for reasons we do not understand or perhaps, the anesthesia. Nor have we been able to evoke discharges by stimulation of sensory pathways. There is much variability although most units tend to "fire" irregularly 2-10 ips. The spectrum of types of units and their characteristics will take time to obtain. Under development for the purpose of characterizing these units in a quantitative way is suitable software. It is hoped that we can then label classes of cells which are affected in recognizable ways and provide a basis for determining the effect of ischemia and/or a cold lesion on their functional activity and significance.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02720-03 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stress Protein Induction in Gerbil Brain After Ischemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T.S. Nowak, Jr. Expert LNNS, NINDS

Others: J. Ikeda Visiting Fellow LNNS, NINDS  
 F. Joo Visiting Scientist LNNS, NINDS  
 A.M. Marini Staff Fellow CNB, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

|                  |     |               |     |        |     |
|------------------|-----|---------------|-----|--------|-----|
| TOTAL MAN-YEARS: | 0.5 | PROFESSIONAL: | 0.5 | OTHER: | 0.0 |
|------------------|-----|---------------|-----|--------|-----|

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major stress/heat shock protein, hsp70, is strikingly induced in hippocampal circuitry following ischemia. Current *in situ* hybridization results show that, while hsp70 mRNA is transiently expressed in dentate granule cells and CA3 neurons which also accumulate immunoreactive hsp70, the mRNA is even more strikingly elevated in CA1 neurons which fail to express the protein, disappearing only with the onset of cell death in this vulnerable population. These results are consistent with the possibility that hsp70, like the proto-oncogene *c-fos*, may provide a physiological marker for some aspect of neuronal activation under pathological conditions. Preliminary studies with a *c-fos* probe show a characteristically transient induction in dentate granule cells after ischemia, indicating that it strongly labels only a subset of the cell populations which eventually express the mRNA for hsp70.

Hyperthermia results in a largely glial hsp70 induction, except in cerebellum. Immunocytochemical studies at the electron microscopic level confirm the presence of hsp70 in cerebellar granule cell neurons after hyperthermia, while verifying the glial nature of positive cells in other brain regions.

|   |                 |  |
|---|-----------------|--|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                 | <b>PROJECT NUMBER</b><br><br>Z01NS 02721-03 LNNS |
| <b>PERIOD COVERED</b><br>October 1, 1988 to September 30, 1989  |                 |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Regulation of Hippocampal Dynorphin Levels and Synthesis After Ischemia   |                 |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |                 |  |
| PI:   | T.S. Nowak, Jr. | Expert LNNS, NINDS                               |
| Other:  | J. Ikeda, M.D.  | Visiting Fellow LNNS, NINDS                      |
| <b>COOPERATING UNITS</b> (if any)<br>Jacqueline McGinty, East Carolina University, School of Medicine   |                 |  |
| <b>LAB/BRANCH</b><br>Laboratory of Neuropathology and Neuroanatomical Sciences  |                 |  |
| <b>SECTION</b><br>Section of Cerebrovascular Pathology  |                 |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892   |                 |  |
| TOTAL MAN-YEARS:  | 0.3             | PROFESSIONAL: 0.3      OTHER: 0.0                |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>   |                 |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p>In situ hybridization studies demonstrate a decrease in prodynorphin mRNA in dentate granule cells of gerbil hippocampus following transient ischemia. Quantitative assessment by means of grain counts following autoradiography indicate a decrease of approximately 50% at 24 h recirculation, comparable to the decreases in dynorphin peptide immunoreactivity in hippocampal extracts observed in previous studies. This down-regulation of dynorphin synthesis at the transcriptional level is similar to that described by other investigators following seizures, and is consistent with an increased activation of hippocampal circuitry following ischemia. In view of the concurrent induction of c-fos and hsp70 in this cell population, altered synthesis of opioid peptide precursors would appear to be another component of what is coming to be understood as a complex pathophysiological response of neurons to ischemia and other stresses.</p> |                 |  |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02722-03 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparison of Hydrogen Clearance and Tracer Diffusion Methods for Determining Cerebral Blood Flow

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T.S. Nowak, Jr. Expert LNNS/NINDS

Other: J. Ikeda Visiting Fellow LNNS/NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated. The final results appear in J Cerb Blood Flow Metab, 1989; 9: 79-86.

|  |                          |   |
|--|--------------------------|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                          | <b>PROJECT NUMBER</b><br><br>Z01 NS 02749-03 LNNS |
| <b>PERIOD COVERED</b><br>October 1, 1988 to September 30, 1989   |                          |   |
| <b>TITLE OF PROJECT</b> <small>(80 characters or less. Title must fit on one line between the borders.)</small><br>The Measurement of Cerebral Blood Flow by Laser Doppler Velocimetry   |                          |   |
| <b>PRINCIPAL INVESTIGATOR</b> <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small>  |                          |   |
| PI:  | H.G. Wagner              | Scientist Emeritus      LNNS, NINDS               |
| Other:   | S. Xu                    | Visiting Fellow      LNNS, NINDS                  |
| <b>COOPERATING UNITS</b> <small>(if any)</small><br>Biomedical Engineering, Research Services, NIH, R.F. Bonner  |                          |   |
| <b>LAB/BRANCH</b><br>Laboratory of Neuropathology and Neuroanatomical Sciences   |                          |   |
| <b>SECTION</b><br>Section of Neurocytobiology  |                          |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892  |                          |   |
| <b>TOTAL MAN-YEARS:</b> 1.2  | <b>PROFESSIONAL:</b> 1.0 | <b>OTHER:</b> 0,2                                 |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>   |                          |   |
| <b>SUMMARY OF WORK</b> <small>(Use standard unreduced type. Do not exceed the space provided.)</small><br><br>A study has been made of cerebral blood flow (CBF) in gerbils by laser Doppler velocimetry (LDV). The gerbils were subjected to repeated bilateral carotid occlusions and studied for up to 3 hours. Continuous measurement of the CBF was made of both parietal cortices simultaneously using 2 probes separately located. The purpose of these experiments was to examine the agreement between the two sides. The essential finding was that while the CBF dynamics were usually similar, they were not always the same and the differences could be substantial in time of recovery, magnitude of recovery and in pattern of recovery. |                          |   |



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02751-03 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cultures of Mouse Capillary Endothelium: Establishment, Growth and Characterization\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Spatz Section Chief LNNS, NINDS

Others: R. M. McCarron Senior Staff Fellow LNNS, NINDS  
L. Wang Guest Researcher LNNS, NINDS

## COOPERATING UNITS (if any)

Dale E. McFarlin, CNP, NIB, NINDS

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Neurocytobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.7

## PROFESSIONAL:

0.2

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study represents a continuous effort to develop and establish the most reliable and reproducible method for culturing cerebromicrovascular endothelium derived from SJL mice susceptible to EAE.

The focus of the present investigation has been to analyze the nutritional requirements needed for sustained long-term growth and propagation of the endothelium. High concentration of endothelial cells grow equally well in medium containing 10-20% of fetal calf serum (FCS). They required endothelial cell growth factor (ECGF) for maximal proliferation. Inclusion of heparin synergistically increased the proliferative response of the cells. In the presence of high concentration of FCS (20%) heparin surpassed ECGF in the ability to support increased proliferative response of the endothelium. A more detailed analysis of various nutrients is still required for establishment and characterization of long-term endothelial culture for expediting the investigation concerned with interaction between capillary endothelium and immune cells.

\*Formerly: "Interaction Between Cerebral Capillary Endothelium and Immune Cells".

## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02764-02 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Intravascular Volume After Repeated Ischemic Insults

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Pluta Guest Researcher LNNS, NINDS

Others: T.S. Nowak, Jr. Expert LNNS, NINDS  
J. Ikeda Visiting Fellow LNNS, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated. The final results appear in J Cereb Blood Flow Metab 1989; 9: 163-70.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02768-02 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Postischemic Accumulation of Calcium in Brain Tissue\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |                    |             |
|---------|-----------------|--------------------|-------------|
| PI:     | T.S. Nowak, Jr. | Expert             | LNNS, NINDS |
| Others: | J. Ikeda        | Visiting Fellow    | LNNS, NINDS |
|         | G. Nagashima    | Visiting Fellow    | LNNS, NINDS |
|         | F. Joo          | Visiting Scientist | LNNS, NINDS |
|         | J. Lohr         | Lab. Technician    | LNNS, NINDS |
|         | C. Ruetzler     | Biologist          | LNNS, NINDS |
|         | I. Klatzo       | Chief              | LNNS, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section on Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.6

## PROFESSIONAL:

1.4

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Observations on the distribution and the time sequences of calcium accumulation following single and repetitive ischemic insults using 45 calcium autoradiography revealed significant differences in the pattern of calcium uptake between single and repeated occlusions. A striking feature of calcium accumulation in repeated ischemias was intense uptake of  $\text{Ca}^{++}$  in thalamus, striatum and, later, in the medial geniculate and substantia nigra. Also, a striking finding was an intense  $\text{Ca}$  uptake in CA1 when the pyramidal neurons were virtually destroyed. Our observations suggest that abnormal accumulation of calcium may be due to other causes than a direct ischemic injury and may not necessarily indicate an irreversible neuronal damage.

\*Formerly: "Post-Ischemic Accumulation of Calcium in Brain Tissue and Histological Ischemic Injury".

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02773-02 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Observations on Global Cerebral Ischemia in Rats

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |                    |             |
|---------|-----------------|--------------------|-------------|
| PI:     | G. Nagashima    | Visiting Fellow    | LNNS, NINDS |
| Others: | N. Saito        | Visiting Fellow    | LNNS, NINDS |
|         | T.S. Nowak, Jr. | Expert             | LNNS, NINDS |
|         | G. Mies         | Visiting Associate | LNNS, NINDS |
|         | J. Lohr         | Lab. Technician    | LNNS, NINDS |
|         | C. Ruetzler     | Biologist          | LNNS, NINDS |
|         | I. Klatzo       | Chief              | LNNS, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section on Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

1.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Global cerebral ischemia is produced in rats by compression of major cardiac vessels, simulating condition of cardiac arrest in man. Postischemic changes are studied in various parameters of injury in order to get insight into nature of chronic, postischemic neuronal changes, in order to devise means of reducing or avoiding a postischemic injury.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02775-01 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Blood -Brain Barrier Changes Following Repeated Ischemic Insults

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |                    |             |
|---------|-----------------|--------------------|-------------|
| PI:     | T.S. Nowak, Jr. | Expert             | LNNS, NINDS |
| Others: | F. Joo          | Visiting Scientist | LNNS, NINDS |
|         | J. Ikeda        | Visiting Fellow    | LNNS, NINDS |
|         | G. Nagashima    | Visiting Fellow    | LNNS, NINDS |
|         | N. Saito        | Visiting Fellow    | LNNS, NINDS |
|         | C. Ruetzler     | Biologist          | LNNS, NINDS |
|         | I. Klatzo       | Chief              | LNNS, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.8

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rabbit antiserum have been raised against purified gerbil albumin and used for the immunocytochemical localization of the extravasated protein in brain following repeated ischemic results. A striking accumulation of albumin immunoreactivity was evident in thalamus as early as 6 h after a series of repeated 5 min carotid artery occlusions while positive staining was first evident in this region at 24 h following repeated 2 min occlusions. At longer recirculation intervals thalamic staining was intensified and the CA1 region of hippocampus showed strong immunoreactivity which accompanied the degeneration of neurons in this vulnerable area. In general, albumin immunoreactivity was closely correlated with the distribution of enhanced calcium-45 uptake as well as with the appearance of reactive glia, as evidenced by immunocytochemical visualization of glial fibrillary acidic protein. Ongoing electron microscopic studies are focused on the cellular distribution of extravasated albumin during the maturation of ischemic injury.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02776-01LNNS

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Production of Experimental Allergic Encephalomyelitis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.M. McCarron Senior Staff Fellow LNNS, NINDS

## COOPERATING UNITS (if any)

Dale McFarlin, Chief, NIB, NINDS  
Bob Fallis, Medical Staff Fellow, NIB, NINDS

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Neurocytobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.5

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In SJL, PL, and SJLxPL (F1) mice, the induction of chronic relapsing murine experimental allergic encephalomyelitis (EAE) by the passive transfer of guinea pig myelin basic protein (MBP)-immune T cells requires the in vitro proliferation of these cells in the presence of both antigen (MBP) and antigen-presenting cells (APC). After transfer, all mice demonstrated clinical signs of neurological dysfunction and pathological analysis revealed an inflammatory response in the CNS accompanied by primary demyelination. All mice recovered and developed a chronic relapsing-remitting form of disease. Although it is known that MBP and MHC class II (Ia) antigen-restricted T cells are required for the induction of EAE, little is known regarding the antigenic specificities and class II restriction requirements of T cells responsible for subsequent relapses and remissions. The purpose of this work is to examine the antigenic specificities and Class II restriction requirements of T cells isolated from mice with chronic relapsing EAE and to determine if they play a role in this disease model.

Specific encephalitogenic MBP epitopes, which differ according to class II phenotype, are required for the induction of EAE in susceptible animals. MBP residues 90-101 are encephalitogenic in SJL mice (H-2<sup>s</sup>) while residues 1-9 are encephalitogenic in PL mice (H-2<sup>d</sup>). In the acute EAE model in which disease is induced by active immunization with antigen, only the MBP fragment including residues 1-9 is encephalitogenic in SJLxPL (F1) mice (i.e. the response is not codominant). However, in the chronic relapsing EAE model where disease is produced by the passive transfer of immune T cells, both of the above determinants were encephalitogenic in F1 mice. Utilizing purified F1 T cells from mice immunized with either MBP fraction 1-37 or 89-169 and cultured in the presence of either PL(H-2<sup>d</sup>) or SJL (H-2<sup>s</sup>) APC respectively, EAE was induced in F1 mice by passive transfer. It was observed that during the course of chronic relapsing EAE, new T cell antigen specificities and class II restriction requirements were generated. This indicates the possibility that antigens distinct from those required to induce EAE may be involved in the onset of subsequent relapses during the course of disease.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02777-01 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Cerebromicrovascular Endothelium: Studies in Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Bacic Visiting Fellow LNNS, NINDS

Others: R.M. McCarron Senior Staff Fellow LNNS, NINDS  
M. Spatz Section Chief LNNS, NINDS

## COOPERATING UNITS (if any)

The Johns Hopkins Hospital, Baltimore, MD (Donlin Long and Sumio Uematsu)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Neurocytobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.8

## PROFESSIONAL:

0.5

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human  
Tissue☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The establishment of endothelial cell (EC) cultures derived from three fractions of microvessels of human brain permitted so far a limited characterization of EC receptors linked to adenylate cyclase (AC). These investigations indicate that the endothelial AC system is responsive to a variety of vasoactive substances: adrenergic, dopaminergic, serotonergic, PGD<sub>2</sub> and PGE<sub>2</sub> (all at conc 10<sup>-5</sup>M), irrespective of its origin. The sensitivity of AC to adrenergic agents was greater in the capillary endothelium than in arteriolar EC. The AC responsiveness to dopamine, 5-HT, PGD<sub>2</sub> and PGE<sub>2</sub> was greatest in EC derived from small arterioles and venules. Prostaglandins F-type (10<sup>-5</sup>M) stimulated AC activity of the endothelium derived from arterioles and venules while bradykinin (10<sup>-5</sup>M) enhanced the activity of capillary and small arteriolar ECs. The enhancement of AC activity to adrenergic substances could be inhibited by butoxamine (β<sub>2</sub>-blocker) and prazosin (α<sub>1</sub>-blocker) indicating the presence of β<sub>2</sub>- and α<sub>1</sub>-adrenergic receptors linked to AC in all EC fractions. The presence of D<sub>1</sub>- but not D<sub>2</sub>-receptors was indicated by inhibition of dopamine AC response with D<sub>1</sub>- but not D<sub>2</sub>-antagonists [SCH-23390, R(+) and sulpiride, S(-), respectively]. The presence of 5-HT<sub>1</sub>-receptors linked to AC was suggested by mianserin (5-HT<sub>1</sub> > 5-HT<sub>2</sub> antagonist) inhibition of 5-HT effect on AC activity and ineffectiveness of α-methylserotonin (5-HT<sub>2</sub> agonist).

This study represents a first demonstration of cultured human cerebral endothelium derived from microvessels of different sizes showing not only the same but different receptors linked to AC activity.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02780-02LNNS

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Vascular Endothelial Cell-Specific Monoclonal Antibodies \*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |               |                     |             |
|---------|---------------|---------------------|-------------|
| PI:     | R.M. McCarron | Senior Staff Fellow | LNNS, NINDS |
| Others: | M. Spatz      | Section Chief       | LNNS, NINDS |
|         | J. Bembry     | Biologist           | LNNS, NINDS |
|         | N. Merkel     | Chemist             | LNNS, NINDS |

## COOPERATING UNITS (if any)

Dale McFarlin, Chief, NIB, NINDS  
 Laura Meuhl, Lab Technician, NIB, NINDS

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Neurocytobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.6

## PROFESSIONAL:

0.6

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To study the blood-brain barrier and its role in various neurological phenomena, endothelial cells (EC) were isolated, purified and cultivated from murine cerebral microvessels. In order to facilitate the study of EC and to identify subpopulations of cerebrovascular EC and determine their biological functions, monoclonal antibodies (mAb) were produced using hybridoma technology. The experiments used to characterize these mAbs included immunofluorescence microscopy, fluorescence activated cell sorter (FACS) analysis, and enzyme-linked immunosorbent assay (ELISA) using cultured brain EC (this assay system was specifically developed to evaluate and characterize these mAbs). Some of these antibodies were quite specific and reacted only with cerebrovascular EC isolated from mice. Additional mAbs had broad reactivity patterns and stained cerebral vascular EC isolated from other animals, as well as EC from other tissues (non-CNS). In regard to using murine cerebral capillary EC, the mAbs exhibited a wide range of reactivity with respect to the types and percentages of cells stained. Populations of freshly isolated EC and cultured EC could be subdivided according to their ability to stain positively with one or more mAbs or to be not stained at all. The work regarding the development and characterization of these antibodies has recently been submitted for publication and should prove useful to various investigators interested in examining differentiation markers of EC as well as EC function.

\* Formerly: "Cerebrovascular Endothelial Antibodies."

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02795-01LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Cerebromicrovascular Endothelial Culture: Cholinergic and Histaminergic Receptors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Spatz Section Chief LNNS, NINDS

Others: F. Bacic Visiting Fellow LNNS, NINDS  
R.M. McCarron Senior Staff Fellow LNNS, NINDS

## COOPERATING UNITS (if any)

The Johns Hopkins Hospital, Baltimore, MD, (Donlin Long and Sumio Uematsu)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Neurocytobiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.6

## PROFESSIONAL:

0.3

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Acetylcholine and histamine are known as vasoactive substances which may be involved in regulating cerebral blood flow (CBF) and/or blood-brain barrier (BBB) functions. This supposition has been supported by detection of cholinergic and histaminergic fibers in the close vicinity of cerebral vessels. Moreover, receptors for both substances were described in microvessels isolated from brain of animals but not from man. The aim of this investigation was to examine the effect of acetylcholine, carbachol and histamine on adenylate cyclase (AC) activity in the endothelium derived from three fractions of human microvessels.

The response of adenylate cyclase to acetylcholine, carbachol and histamine was seen in all examined homogenates of endothelium (4<sup>th</sup>-11<sup>th</sup> passage) derived from large microvessels. Pirenzepine (M<sub>1</sub>-muscarinic inhibitor) blocked the acetylcholine and carbachol stimulated formation of cAMP while methoctramine (M<sub>2</sub> muscarinic inhibitor) had no effect on AC of endothelium derived from large microvessels. Preliminary investigations demonstrated a dose-dependent cholinergic stimulation of cAMP production in endothelium derived from capillaries and small microvessels. The response of endothelial AC activity to histamine was inhibited (30-100%) by cimetidine indicating the AC linkage to H<sub>2</sub>-receptors. These findings represent the first demonstration of cholinergic and histaminergic receptors linked to AC system in cerebromicrovascular endothelium of human brain.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02796-01 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transneuronal Effects of Cryogenic Brain Injury on Calcium Uptake and Blood-Brain Barrier Changes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |                    |             |
|---------|-----------------|--------------------|-------------|
| PI:     | J. Ikeda        | Visiting Fellow    | LNNS, NINDS |
| Others: | F. Joo          | Visiting Scientist | LNNS, NINDS |
|         | T.S. Nowak, Jr. | Expert             | LNNS, NINDS |
|         | G. Mies         | Visiting Associate | LNNS, NINDS |
|         | J. Lohr         | Lab. Technician    | LNNS, NINDS |
|         | C. Ruetzler     | Biologist          | LNNS, NINDS |
|         | I. Klatzo       | Chief              | LNNS, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section on Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

1.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A development of transneuronal, secondary, injuries developing in distant but anatomically connected regions following primary, direct injury to the cerebral cortex was shown to be associated with an abnormal uptake of calcium, and BBB permeability changes in those areas. The delayed onset of these changes, pattern of calcium uptake into dendritic structures and intracytoplasmic uptake of extravasated albumin suggest a neuroexcitatory nature of mechanisms involved. If correct, studies will be developed to ascertain whether suppression or reduction of neuroexcitation by some agents could substantially ameliorate or prevent development of secondary transneuronal injuries. This could be of relevance to the treatment of brain trauma patients.



|  |                          |   |
|--|--------------------------|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                          | <b>PROJECT NUMBER</b><br><br>Z01 NS 02797-01 LNNS                     |
| <b>PERIOD COVERED</b><br>October 1, 1988 to September 30, 1989   |                          |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Cultures of Human Cerebromicrovascular Endothelium: Establishment, Growth and Characterization   |                          |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)   |                          |   |
| PI:  | M. Spatz                 | Section Chief<br>LNNS, NINDS  |
| Others:  | R.M. McCarron<br>L. Wang | Senior Staff Fellow<br>Guest Researcher<br>LNNS, NINDS<br>LNNS, NINDS |
| <b>COOPERATING UNITS</b> (if any)<br>Drs. Donlin Long and Sumio Uematsu, The John Hopkins Hospital, Baltimore, MD<br>Dr. Ronald F. Dodson, University of Texas, Health Center, Tyler, TX   |                          |   |
| <b>LAB/BRANCH</b><br>Laboratory of Neuropathology and Neuroanatomical Sciences   |                          |   |
| <b>SECTION</b><br>Section of Neurocytobiology  |                          |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892  |                          |   |
| TOTAL MAN-YEARS:   | 0.9                      | PROFESSIONAL: 0.5<br>OTHER: 0.4                                       |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>  |                          |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p>             This study has been concerned with the development and establishment of endothelial cell cultures derived from three microvascular fractions of human brain. The purity of the endothelial cultures was found greater than 95% since most of the cells expressed human Factor VIII-related antigen and only occasional cell stained for glial fibrillary acidic protein. Both light and electronmicroscopy showed that the separately cultured endothelium derived either from the capillaries (EC) or small [<math>&lt;100\mu\text{m}</math>] ES and large [<math>&gt;100\mu\text{m}</math>] EL arterioles and venules exhibited the same appearance and growth pattern. The maximal proliferative response of endothelium was seen on the first day after exposure to fresh medium. A direct relationship was found between various components of growth medium and individual proliferative response of endothelium dependent on its origin indicating different growth requirements. The characterization of the cultured endothelium is still incomplete and in progress.           </p> <p>             Nevertheless the successfully developed technique for cultivating pure cerebromicrovascular endothelium derived from human brain (dependent on the availability of brain tissue) provides a model <u>in vitro</u> for investigation of endothelial properties related to the normal or altered function of blood-brain barrier and cerebral blood flow in man.           </p> |                          |   |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02798-01 LNNS

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ultrastructural Observations on Distribution of Calcium in Cerebral Ischemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |              |                    |             |
|---------|--------------|--------------------|-------------|
| PI:     | F. Joo       | Visiting Scientist | LNNS, NINDS |
| Others: | J. Ikeda     | Visiting Fellow    | LNNS, NINDS |
|         | G. Nagashima | Visiting Fellow    | LNNS, NINDS |
|         | J. Lohr      | Lab. Technician    | LNNS, NINDS |
|         | C. Ruetzler  | Biologist          | LNNS, NINDS |
|         | I. Klatzo    | Chief              | LNNS, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section of Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.7

## PROFESSIONAL:

1.6

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

|   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using the pyroantimonate method for precipitating calcium to electron-dense calcium pyroantimonate, the ultrastructural distribution of calcium was evaluated following single and repeated ischemic insults, produced by bilateral occlusions of common carotid artery in gerbils. Our observations revealed an excessive accumulation of calcium deposits in the mitochondria of pyramidal cell dendrites preceding their destruction. Following the disappearance of pyramidal cells, which becomes evident approximately 3 days after ischemic insults, a striking accumulations of electron dense calcium deposits was observed in the dendrites of the interneurons, whereas mitochondria of these dendrites were free of calcium. Occasionally the reverse was true and calcium deposits were conspicuous in the center of mitochondria with well preserved cristae, whereas there was no calcium in the cytosol of the dendrites. The pattern of calcium distribution in the dendrites appears similar to that observed following convulsive seizures and indicates a possibility of neuroexcitatory mechanisms playing an important role in accumulation of calcium in injured by ischemia neurons. Otherwise, the intraneuronal presence of calcium itself does not seem to be invariably associated with a neuronal death.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

**PROJECT NUMBER**

Z01 NS 02801-01 LNNS

**PERIOD COVERED**

October 1, 1988 through September 30, 1989

**TITLE OF PROJECT** (80 characters or less. Title must fit on one line between the borders.)

Interactions Between Cerebrovascular Endothelial Cells and Immune Lymphocytes

**PRINCIPAL INVESTIGATOR** (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. R. M. McCarron Senior Staff Fellow LNNS, NINDS

Other: M. Spatz Section Chief LNNS, NINDS

**COOPERATING UNITS** (if any)

Dale McFarlin, Chief, NIB, NINDS  
 Michael Rache, Senior Staff Fellow, NIB, NINDS

**LAB/BRANCH**

Laboratory of Neuropathology and Neuroanatomical Sciences

**SECTION**

Section of Neurocytobiology

**INSTITUTE AND LOCATION**

NINDS, NIH, Bethesda, Maryland 20892

**TOTAL MAN-YEARS:**

0.4

**PROFESSIONAL:**

0.4

**OTHER:**

0

**CHECK APPROPRIATE BOX(ES)**

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

**SUMMARY OF WORK** (Use standard unreduced type. Do not exceed the space provided.)

The cerebral capillary endothelial cell (EC) is strategically located to play a critical role(s) in the regulation of cell trafficking from the peripheral blood into the central nervous system (CNS). Studies regarding the interaction between cerebral capillary EC and immune lymphocytes may illuminate our understanding of mechanisms leading to the breakdown of the blood-brain barrier (BBB) and the vascular egress of immune lymphocytes and other peripheral blood elements into the CNS, which is a hallmark of neuroimmunological disorders such as Multiple Sclerosis and the model disease Experimental Allergic Encephalomyelitis (EAE).

The experiments performed here were designed to study the specific interaction between cerebrovascular EC and myelin basic protein (MBP)-reactive T lymphocytes, which adoptively transfer chronic relapsing EAE in naive syngenic mice. The capacity of cerebral capillary EC to serve as targets for these T lymphocytes [which can function as cytotoxic T lymphocytes (CTL)] is being examined. The kinetics of the CTL-mediated lysis of EC targets follow classical fashion (i.e. direct relationship between percent specific lysis and effector:target ratio). The CTL response was class II MHC-restricted and required the expression of Ia antigen on the target (EC) surface. Anti-Ia antibody treatment of targets blocked the lytic destruction; also, Ia-negative EC were not affected. In addition, only syngenic combinations of EC targets and CTL's resulted in lysis. The CTL response was also antigen-specific; EC targets incubated in the absence of antigen or pulsed with irrelevant antigen were unaffected. In addition to functioning as targets, EC also interacted with lymphocytes during the generation of proliferative responses. Although EC could function as antigen-presenting cells for purified T cell populations, the addition of EC to whole immune lymph node cell populations resulted in the inhibition of antigen-specific proliferative responses. This inhibition could be reproduced by adding EC culture supernatants and the generation of this inhibitory factor could be blocked by the inclusion of indomethacin in EC cultures. Analysis of EC supernatants indicated the presence of high (inhibitory) concentrations of various prostaglandin species (PGI as well as PGE).

These data indicate that EC-lymphocyte interactions may provide potential mechanisms for the pathological alteration of the BBB, resulting in vascular egress and neuropathology observed in autoimmune diseases such as Multiple Sclerosis.

|   |                     |   |
|---|---------------------|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                     | <b>PROJECT NUMBER</b><br>Z01 NS 02802-01 LNNS                   |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989   |                     |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Immune Mechanisms: Regulation of Ia Antigen Expression  |                     |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |                     |   |
| PI:   | R. M. McCarron      | Senior Staff Fellow<br>LNNS, NINDS                              |
| Others:   | M. Spatz<br>L. Wang | Section Chief<br>Guest Researcher<br>LNNS, NINDS<br>LNNS, NINDS |
| <b>COOPERATING UNITS</b> (if any)<br>Masami Tanaka, Visiting Fellow, NIB, NINDS<br>Elliot Cowan, Special Expert, NIB, NINDS   |                     |   |
| <b>LAB/BRANCH</b><br>Laboratory of Neuropathology and Neuroanatomical Sciences  |                     |   |
| <b>SECTION</b><br>Section of Neurocytobiology   |                     |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892   |                     |   |
| <b>TOTAL MAN-YEARS:</b>   | 1.4                 | <b>PROFESSIONAL:</b> 1.4<br><b>OTHER:</b> 0                     |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><input type="checkbox"/> (a) Human subjects<br><input type="checkbox"/> (a1) Minors<br><input type="checkbox"/> (a2) Interviews<br><input checked="" type="checkbox"/> (b) Human tissues<br><input type="checkbox"/> (c) Neither  |                     |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p>The mechanisms responsible for the induction of experimental allergic encephalomyelitis (EAE) are being examined utilizing SJL mice. This model autoimmune disease is characterized clinically by severe hind limb paralysis and pathologically by the presence of an inflammatory response in the central nervous system (CNS) and resulting primary demyelination. The histopathological observation of perivascular infiltration of inflammatory cells into the CNS has led to research concerning the mechanism by which these cells from the systemic circulation migrate across the blood-brain barrier which is composed of <u>cerebrovascular endothelial cells (EC)</u> forming tight junctions. In addition, all mice in this animal model system recover and develop a chronic relapsing-remitting course of disease. The mechanisms responsible for these relapses and remissions are unknown. Current investigations are focused on the expression and modulation of class II MHC (Ia) molecules on EC as well as <u>CNS-derived astrocytes and macrophages</u>. The capacity of these cells to express Ia molecules and to function as antigen-presenting cells is being studied for their possible role in this disease model. <u>Human cerebrovascular EC</u> isolated from fresh brain tissue surgically removed for treatment of Idiopathic Epilepsy were also tested for their ability to express Ia antigen upon treatment with interferon-gamma (IFN<math>\gamma</math>).</p> <p><u>All cell types could be induced to express Ia antigens by treatment with IFN<math>\gamma</math>. The level of expression and the kinetics of induction differed for each cell population. All Ia-positive cells were also able to function as competent antigen-presenting cells. The mechanism of Ia antigen induction by IFN<math>\gamma</math> treatment of above cells is being examined by culturing cells in the presence of various compounds which were found to modulate Ia expression.</u> Tumor necrosis factor (TNF) augmented the level of Ia expression on astrocytes and inhibited Ia expression on EC, indicating differences between these cells regarding the mechanisms of modulation. The addition of adrenergic agonists such as isoproterenol to EC cultures resulted in significant inhibition in IFN<math>\gamma</math>-induced Ia expression. Experiments utilizing a variety of agonists as well as antagonists were performed to characterize this effect. Simultaneous experiments examining the level of cAMP formation in EC showed that isoproterenol augmented the level of cAMP. <u>The correlation between cAMP formation and Ia expression indicates a potential interaction between the pathways leading to their formation.</u></p> |                     |   |



|   |   |  |
|---|---|--|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |   | PROJECT NUMBER<br><b>Z01 NS 02750-03</b><br>LNNS   |
| PERIOD COVERED<br>October 1, 1988 through September 30, 1989  |   |  |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)<br><b>Observations on Cerebral Ischemia at Injury Threshold Levels.</b>   |   |  |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>PI: <b>M. Seida, MD Visiting Fellow, LNNS, NINDS</b>   |   |  |
| COOPERATING UNITS (if any)  |   |  |
| LAB BRANCH<br><b>Laboratory of Neuropathology &amp; Neuroanatomical Sciences, BNP</b>   |   |  |
| SECTION<br><b>Section on Cerebral Pathology</b>   |   |  |
| INSTITUTE AND LOCATION<br><b>NINDS, NIH, Bethesda, MD 20892</b>   |   |  |
| TOTAL MAN-YEARS:<br><div style="text-align: center;">0</div>  | PROFESSIONAL:<br><div style="text-align: center;">0</div> | OTHER:<br><div style="text-align: center;">0</div> |
| CHECK APPROPRIATE BOX(ES)<br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div> |   |  |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)<br><b>This project was terminated 10/88.</b>   |   |  |







# ANNUAL REPORT

October 1, 1988 through September 30, 1989  
Laboratory of Neurophysiology  
Basic Neurosciences Program, DIR  
National Institute of Neurological  
Disorders and Stroke

|   |       |
|---|-------|
| Research Summary  | II-IV |
| Project Reports   |       |
| Physiological Properties Developing on CNS Cells<br>Z01 NS 02019-17 LNP                                   | 1     |
| Cell Biological Studies of Developing CNS Cells<br>Z01 NS 02330-12 LNP                                    | 2     |
| Synaptic Contacts of Retinal Neurons<br>Z01 NS 01659-21 LNP   | 3     |
| Structure and Function in Retinal Neurons<br>Z01 NS 02631-06 LNP  | 4     |
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Annual Report  
October 1, 1988 through September 30, 1989

Laboratory of Neurophysiology, BNP, DIR  
National Institute of Neurological Disorders and Stroke  
Jeffery L. Barker, M.D., Chief

The Laboratory of Neurophysiology's research program has been divided primarily between application of relatively well-established techniques in electrophysiology for the purpose of generating new insight into intercellular communication and signal transduction mechanisms and the development of new strategies in physiological recording techniques to increase the rate at which such insights can be obtained. Most, if not all, of the research endeavors are related to the physiology of intercellular communication and signal transduction mechanisms expressed by vertebrate CNS cells.

The long-term goal of this program is to elucidate details regarding the development, differentiation and cellular distribution of specific circuits in vertebrate nervous systems. All cells exhibit receptors, ion channels and signal transduction mechanisms that underlie all types of intercellular communication. How, when and where these develop in vertebrate nervous systems is the focus of much of our research activities. Multi-disciplinary study of the differentiation of receptors, ion channels and signal transduction mechanisms during development should reveal fundamental insights into the process of distributing such mechanisms among cells and their roles both in differentiating and in differentiated circuits. In time, we aim to provide a reference for studying specific pathophysiology of the vertebrate CNS.

All of the projects are at cellular or molecular levels of study. Experimental substrates and lines of investigation include: 1) monolayers of primary nerve and glial cells cultured from embryonic and early post-natal mammalian and avian central nervous systems; 2) monolayers of cultured fibroblasts transfected with genes encoding specific transmitter receptors; 3) acutely isolated neurons from embryonic and well-differentiated CNS tissues; 4) retinal eye cup and intact retina; 5) quantitative electrophysiological analysis of cellular excitability expressed in short (hours) or long-term (days-weeks) cultured cells; 6) quantitative electrophysiological analysis of cellular excitability resident in retinal circuits; 7) flow cytometric analysis of physiological properties exhibited in populations of cells from the embryonic vertebrate CNS and fibroblasts transfected with transmitter receptor genes; 8) light- and electron-microscopic resolution of cellular form and subcellular structure in normally developed mammalian CNS tissues including retina and spinal cord; 9) immunohistochemical characterization of specific transmitter phenotype expressions *in vivo* and in monolayer culture; and 10) quantitative analysis of optical signals emitted by vital embryonic cells or associated with the morphology of differentiating glia and neurons. Conceptually, these diverse strategies and lines harmonize to allow quantitative resolution of receptor functions, ion channel expressions and signal transduction mechanisms. The strength of the Laboratory's research enterprise lies in the spectrum of contemporary and innovative strategies at single-cell, circuit and population levels, and the opportunity for multi-disciplinary and collaborative study of basic problems into the physiology of intercellular communication emerging in the embryonic CNS.

Considerable effort has been applied during FY 1989 to the further development of optical recording techniques at single-cell and population levels. We now have several optical recording systems arranged to acquire physiological signals (membrane potential, intracellular ions) with two of these systems having the added capability of simultaneous recording of both electrical and optical events. The latter are especially critical since they can be used to compare, in a quite direct and quantitative manner, the physiological properties of cells studied with both contemporary electrophysiological recording and optical indicator-dye techniques. Direct comparison of electrical and optical signals at the single-cell level should permit extrapolation to results involving optical recordings of physiologically important properties in completely intact cells.

Several developments this year will have long-lasting consequences:

Dr. Marc Walton, in collaboration with Dr. Anne Schaffner, has developed an optical recording system capable of acquiring physiologically relevant fluorescent indicator dye signals simultaneously from statistically sizable samples of cells. He has applied voltage-sensitive dye to embryonic rat spinal cord cells and probed the emergence of functional  $\text{Na}^+$  channels. His results complement those obtained by Dr. Schaffner and others on the same cell types with flow cytometric techniques and demonstrate that such a strategy provides a powerful new perspective for examining physiological signals in intact cells differentiating for hours to weeks in monolayer culture.

Drs. Schaffner and Anita Prasad have used the monovalent cationophore gramicidin to calibrate the fluorescence intensity emanating from voltage-sensitive dye-stained spinal cord cells in terms of membrane potential. Over the physiological range of membrane potential fluorescence intensity changes some 10-20 fold and most cells in suspension appear well polarized near the equilibrium potential for  $\text{K}^+$  ions.

Ms. Toby Behar and Dr. Schaffner have used immunocytochemistry to demonstrate that embryonic spinal and hippocampal neurons contain the neurotransmitter GABA in the relative absence of the rate-limiting enzyme involved in GABA synthesis in postnatal CNS tissues. GABA may thus be derived from other sources, like polyamines, during embryogenesis and play physiological roles during this period.

Dr. Morris Benveniste has used a relatively novel dual-recording strategy to discover that synaptically-connected cultured rat hippocampal neurons often generate virtually identical GABA-mediated signals at pre- and postsynaptic sites. Perhaps there are subtypes of  $\text{GABA}_A$  receptor expression with different, but relatively uniform properties that correspond to different  $\text{GABA}_A$  receptor subunit compositions.

Drs. Prasad and Schaffner have pioneered the use of dual-dye staining to study membrane potential and intracellular  $\text{Ca}^{2+}$  signals in suspended populations of cells. Simultaneous changes in plasma membrane potential and intracellular  $\text{Ca}^{2+}$  arising from  $\text{Ca}^{2+}$  channels in plasma and endoplasmic reticular membranes can now be detected in entire populations of cells using flow cytometry.



Dr. Thomas Smith and Ms. Behar have utilized fractal analysis techniques to quantify the differentiation of shape complexity among glial elements. The latter differentiate more complex shapes than neurons in several days with an exponential time course.

Dr. Karl Thor has developed an innovative strategy to identify embryonic neurons that project from spinal cord to thalamus. Spinothalamic tract (STT) neurons constitute the second limb of the circuit conveying pain. The multi-step protocol he has developed involves 1) intra-uterine injection of fluorescent dye into the thalamic region of embryonic rats; 2) several days further gestation in utero to allow retrograde labelling of STT neurons in the spinal cord; 3) dissociation of the retrogradely labelled STT neurons into single-cell suspensions; and 4) differentiation of these neurons in monolayer culture for weeks. In time it may be possible to co-culture nociceptive primary afferents with STT neurons and the latter with thalamic target cells in order to investigate the developmental and cellular biology of major components of the pain circuit in the mammalian CNS.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02019-17 LNP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological properties developing on CNS cells\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS. Others (LNP, BNP, DIR, NINDS): A.E. Schaffner, Biologist; M.K. Walton, Senior Staff Fellow; P.A. Sheehy, Staff Fellow; N.L. Silva, NRC Fellow; J.M.H. French-Mullen, NRC Fellow; N.L. Harrison, Fogarty Associate; R. Cruciani, Fogarty Associate; S.V.P. Jones, Fogarty Fellow; P.A. Brooks, Special Volunteer; R.E. Study, Special Volunteer; M. Smith, Special Volunteer; J. Suszkiw, IPA; N. Hardegen, Electronics Technician.

## COOPERATING UNITS (if any)

G.D. Lange (RSB, NINDS); E. Stanley (LB, NINDS); B. Dufy (Lab Neurophysiol., Univ. Bordeaux, France); M.R. Brann (LMB, NINDS)

## LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

8.5

## PROFESSIONAL:..

7.5

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Electrophysiological and optical recording techniques are used primarily to probe the development, differentiation and cellular distribution of physiologically important properties either normally expressed by mammalian CNS cells or genetically engineered in fibroblasts. The quite complementary strategies employed involve patch-pipette recordings of ion fluxes in single cells and synaptically coupled pairs of cells and simultaneous digital imaging microscopy of many cells stained with fluorescent potential-sensitive indicator dye. Principal observations this year include 1) fibroblasts transfected with genes encoding for m1 and m3 muscarinic receptors express cholinergic receptors that sequentially stimulate phospholipase C, phosphatidyl inositol turnover, the production of 1,4,5-inositol trisphosphate, rapid mobilization of  $\text{Ca}^{2+}$  from intracellular stores and activation of  $\text{K}^{+}$  conductance; 2) pertussis-toxin insensitive GTP-binding proteins are involved in these responses; 3) embryonic GABAergic neurons cultured from the rat hippocampus often express two forms of auto-inhibition involving  $\text{GABA}_A$  and  $\text{GABA}_B$  receptors; 4) synaptically activated  $\text{GABA}_A$  receptors of functionally coupled neurons express similar, if not identical electrical properties; 5) activation of  $\text{GABA}_B$  receptors by GABA and  $\text{GABA}_B$  mimetics reduce GABA release by acting at pre-synaptic terminal mechanisms; 6) two major classes of voltage-gated  $\text{Ca}^{2+}$  entry are expressed by acutely dissociated postnatal rat nigrostriatal dopamine neurons; 7) acutely dissociated adult guinea pig CA1 hippocampal neurons exhibit voltage-gated  $\text{Ca}^{2+}$  currents different from peripheral sensory neurons; and 8) the general anesthetic enflurane alters passive membrane properties and enhances  $\text{GABA}_A$  receptor-coupled  $\text{Cl}^{-}$  conductance.

\*[Formerly: "Electrophysiological Studies on Membrane Excitability"]

1-LNP, BNP, DIR

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02330-12 LNP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Biological Studies of Developing CNS Cells

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS. Others (LNP, BNP, DIR, NINDS): U. diPorzio, Visiting Scientist; A.E. Schaffner, Biologist; K. Thor, Senior Staff Fellow; M. Fiszman, Visiting Fellow; A. Prasad, Staff Fellow; K.S. Madden, NRC Fellow; T.N. Behar, Microbiologist; S.V. Smith, Biologist; N. Hardegen, Electronics Technician.; V. Smallwood, Bio. Lab. Technician.

## COOPERATING UNITS (if any)

G.D. Lange (RSB, NINDS); S. Nadi (MNB, NINDS); A. Zuddas (CNB, NINDS); L. Mahan (LCB, NIMH)

## LAB/BRANCH

Laboratory of Neurophysiology

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

10

## PROFESSIONAL:

8

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Flow cytometry and immunocytochemical methods are applied to embryonic rat spinal and supraspinal regions to study the development, differentiation and cellular distribution of specific transmitters and their corresponding receptor-coupled alterations in membrane excitability. At present this multi-disciplinary investigation is focused on elucidation of a developmental calendar outlining the expression of specific transmitters and their functional receptors. Principal observations this year include: 1) calibration of fluorescence signals acquired with FACS technology in terms of membrane potential using the cationophore gramicidin; 2) most embryonic elements in suspension are hyperpolarized near -90mV; 3) a minority of early embryonic rat spinal cord elements are depolarized near 0mV; 4) resting potential of the hyperpolarized cells changes directly according to the extracellular K<sup>+</sup> concentration, as if K<sup>+</sup> ions and cell membrane permeation mechanisms account for membrane potential; 5) Na<sup>+</sup> channels emerge before depolarizing responses to transmitters in spinal and supraspinal regions; 6) GABA<sub>A</sub> receptors depolarize cells near -50mV early in development; 7) functional kainic acid receptors appear to precede other glutamate receptor subtypes; 8) in the rat mesencephalon a novel, apparently hyperpolarizing response appears before depolarizing responses to kainic acid and both are blocked by a relatively specific antagonist, CNQX; 9) in embryonic rat spinal and hippocampal regions GABA can be detected immunocytochemically and biochemically before GAD, its rate-limiting enzyme in the postnatal period; 10) an essential fatty acid analogue of arachidonate containing triple rather than double bonds and arachidonate itself alter potentiometric fluorescence signals in most embryonic rat and chick spinal cord cells and the analogue induces developmental anomalies when introduced in the chick spinal cord; and 11) embryonic spinothalamic neurons, which convey nociceptive sensory input from the spinal cord to the thalamus, can be back-labelled, cultured for days to weeks and identified in vitro.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01659-21 LNP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synaptic Contacts of Retinal Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Lasansky, Head, Unit on Cell Biology, LNP, BNP, DIR, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

## SECTION

Unit on Cell Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1

## PROFESSIONAL:

1

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Whole cell recordings of the responses to light of depolarizing bipolar cells in salamander retinal slices remain stable for periods of up to 30 min when the electrode tips are fire-polished to a diameter of about 0.5  $\mu\text{m}$ . The resulting high (40-50 megohm) access resistance is no hindrance when recordings are performed in the discontinuous current or voltage clamp modes. With this method, the amplitude of the current or voltage responses is decreased by depolarizing and increased by hyperpolarizing currents, but the presumed underlying increase in conductance could not be confirmed by obtaining a reversal of response polarity at a depolarized membrane potential. This problem was thought to be caused by poor space clamp due to the large outward rectification observed. Accordingly, the responses could be consistently reversed at a membrane potential of about 10 mV when 20 mM tetraethylammonium was added to the electrode solution. The same results were obtained with light stimuli of 520 or 680 nm, so that it may be assumed that the rod and cone transmitters have the same action on the synaptic membrane.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02631-06

LNP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function in Retinal Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Ralph Nelson, Physiologist, LNP, NINDS

Others: Andrew P. Mariani, Special Expert, LNP, NINDS; Jan N.M. de Melo, Asso. Prof., UFRJ Brazil; Michael Freed, Staff Fellow, LNP, NINDS; Helga Kolb, Professor, U. of Utah; Renate Pflug, Instructor, U. of Vienna; Eberhart Zrenner, Professor, Lud.-Max.-U., Munich; Steven Baer, Asst. Prof., U. of Arizona, Tempe

## COOPERATING UNITS (if any)

Physiology, University of Vienna, Austria (Renate Pflug); Neurobiology, Federal University at Rio de Janeiro, Brazil (Jan N.M. de Melo); Eye Clinic, Ludwig-Maximilians- University, Munich, FRG (Eberhart Zrenner); Physiology, University of Utah School of Medicine, Salt Lake City (Helga Kolb); Mathematics, Arizona State University, Tempe (Steven Baer)

## LAB/BRANCH

Laboratory of Neurophysiology, DIR, NINDS

## SECTION

Neural Circuitry Unit

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

2.0

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neural organization and neural interactions in mammalian retinas are investigated using intracellular recording and staining techniques, electron-microscopy and pharmacological approaches. In the outer plexiform layers of cat and rabbit retinas the dopaminergic agonist apomorphine depolarizes horizontal cells and both suppresses and delays cone-flicker signals. The effect may mimic the natural suppression of cone signals by dark-adapted rods. A feedback model suggests that horizontal cells, when depolarized either by rods, or extrinsic agents, release a substance that antagonizes cone feed-forward synaptic transmission.

In the inner plexiform layer the electrophysiological responses and morphology of alpha and beta type cat retinal ganglion cells (GC's) are studied using intracellular, HRP-filled microelectrodes. Serial electron-microscopic reconstructions reveal about equal numbers of amacrine (including AII-type) and bipolar cell inputs to OFF beta GC's and mainly amacrine input to OFF-alpha GC's. OFF-alpha cells receive input from only one type of bipolar cell whereas OFF-beta cells receive input from two types. In beta GC's OFF-center receptive-field centers match dendritic fields in spatial extent, whereas center mechanisms occupied only the central 1/3 of dendritic fields in OFF-alpha cells. Investigations of ON-alpha GC's reveal about 2000 chemical synapses distributed in dome-like fashion across the dendritic field. This dome-shaped synaptic weighting contributes to the Gaussian profile of alpha-GC receptive-field sensitivity.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02705-04 LNP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anatomical Studies of Neurons, Neurotransmitters and Neurotransmitter Receptors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

A.P. Mariani, Expert, LNP, BNP, DIR, NINDS

## COOPERATING UNITS (if any)

Federal University of Rio de Janeiro (J.N. Hokoc)

Ohio State University College of Medicine (N.H. Neff and M. Hadjiconstantinou)

## LAB/BRANCH

Laboratory of Neurophysiology

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1

## PROFESSIONAL:

1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two types of tyrosine hydroxylase (TH)-immunoreactive neuron are found in the rhesus monkey, Macaca mulatta, retina. One of these types is immunoreactive for aromatic-L-amino acid decarboxylase (AADC), the other is not. These results suggest that the one population of TH-containing amacrine cells does not process L-dopa to dopamine. Such evidence suggests that L-dopa, the precursor of dopamine may be an endogenous neurotransmitter or modulator in the retina. Electron microscopy of retinal tissue conventionally immunoreacted for TH by the peroxidase-antiperoxidase method revealed synaptic input from amacrine cells at conventional synapses, and bipolar cells at ribbon synapses onto the type 2CA amacrine cells. Curiously, although the synaptic input is comparatively easily found, the output synapses, or synapses of the type 2 CA amacrine cells onto other neuronal elements, are rarely found. Some synapses of the type 2 CA cells onto no nonimmunoreactive amacrine cells have been identified, however. The unusual pattern of synaptic organization, with many identifiable input synapses but few morphologically characterizable output synapses, suggests a currently unconventional role for the type 2CA amacrine cells in the circuitry of the primate retina.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02767-02 LNP

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Image Processing And Analysis of Cellular Structures

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T.G. Smith, Jr., Unit Chief, LNP, BNP, DIR, NINDS.

Others (LNP, BNP, DIR, NINDS): Katheleen Madden, Guest Researcher; T.N. Behar, Technician

## COOPERATING UNITS (if any)

W.B. Marks (LNLC, NINDS), G.D. Lange, (IACS, NINDS), W.H. Sheriff, Jr. (IACS, NINDS); E.A. Neale (LDB, NICHD); Seth Goldstein (BEIB), R. Porter, Monash University, Melbourne, Australia

## LAB/BRANCH

Laboratory of Neurophysiology

## SECTION

Unit on Sensory Physiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.7

## PROFESSIONAL:

3.7

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The concepts of Mandelbrot's fractal geometry have been applied to the structure of individual central nervous system neurons and other cell types grown in tissue culture or from whole animals. Techniques have been developed to measure the "fractal dimension" (FD), which is a measure of complexity of individual cells' structure, with particular reference to the degree of their dendritic branching and the roughness of their borders.

These techniques were calibrated against images of known FD and then employed to measure the unknown FD of individual neurons. The range of FD's in 28 neurons examined was between 1.14, indicating a relatively low complexity, to 1.60, indicating a high complexity.

In addition, we have applied our methods to other cell types (e.g., cat cortical pyramidal neurons, glia, etc.)

Our research on cat cortical pyramidal neurons has shown that different pyramidal neuron types from different areas of the motor cortex have different fractal dimensions, with the largest found in those cells that make up the pyramidal track.

Our work on glia cells has shown that primoidal cells that mature into oligodendrocytes develop larger fractal dimensions and at a faster rate than astrocytes. The most remarkable finding, however, is that the rate of development of the fractal dimension with time can be described remarkably well by a single time constant.





## ANNUAL REPORT

October 1, 1988 through September 30, 1989

Laboratory of Molecular and Cellular Neurobiology

Basic Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989

### Laboratory of Molecular and Cellular Neurobiology National Institute of Neurological Disorders and Stroke

Daniel L. Alkon, M.D. , Chief (Rotating)

The integration of the four Sections of the Laboratory of Molecular and Cellular Neurobiology (LMCN) at the Park Building in Rockville has proceeded smoothly. Productive collaborative research has been undertaken and a continuing program of speakers and laboratory wide discussions have added to the intellectual vitality of a broad variety of scientific programs.

#### Neural Systems Section

The **Neural Systems Section** takes a multidisciplinary approach to the question of how information is stored during associative learning and how it is made available for later recall. Biophysical and molecular mechanisms of associative learning are being analyzed in parallel for a mollusc (the sea snail *Hermisenda crassicornis*), the rabbit, and, most recently, the rat. Parallel analyses offer the important opportunity for uncovering general cellular principles of learning and memory - principles which have been conserved over the course of evolution and which therefore could have relevance for human cognition. Parallel analyses also permit exploitation of critical experimental advantages unique to diverse species. For *Hermisenda* we have been able to demonstrate the first causal relationship of biophysical and molecular transformations within individual neurons to Pavlovian conditioning of a living animal. Causal relationships of cellular physiology and associative learning have not yet been approximated for any vertebrate preparation. Nevertheless we found evidence of biophysical transformations which are common to both mollusc and mammal. An identified group of neurons, the CA1 cells (rather than individual identified neurons) was shown to have a distribution of conditioning-specific modification of K<sup>+</sup> channels within hippocampal slices removed from rabbits on days after they had been conditioned. Such slices provide vastly greater amounts of tissue (than does the snail) for biochemical studies. Indeed, we have already observed conditioning-specific translocation of protein kinase C (PKC) not only in the hippocampus CA1 neurons, but also in a restricted region of the cerebellar cortex called "H6". Similar memory-specific translocation of PKC has now been found in the rat hippocampus. PKC regulation of identical K<sup>+</sup> channels occurs in both *Hermisenda* and hippocampal neurons. In *Hermisenda* this regulation is being pursued with isolated membrane patches whose intracellular surfaces are accessible to precise ionic and biochemical manipulations. The distribution of PKC-mediated neural changes with associative learning in large neuronal arrays is currently being

analyzed with autoradiographic techniques. Within the past year image analyses have revealed changes within neuronal populations of conditioned but not control brain areas. Also recently, PKC regulation has been linked to conditioning-specific modification of *Hermissenda* m-RNA metabolism. This molecular storage step occurs within a specific temporal window during the retention period of the associative memory. Convergence of CS and UCS pathways activated during associative conditioning of the nudibranch mollusc *Hermissenda* involves inhibition of type B photoreceptors by hair cells caudally located in statocysts. Recently we demonstrated that this inhibition is entirely transformed into excitation when stimulation of the visual pathway is precisely timed in relation to stimulation of the vestibular pathway. The pairing-specificity of this vestibular-visual synapse has important implications not only acquisition for associative memory in the snail *Hermissenda* but for mammalian systems as well. Furthermore, theoretical constructs formulated on the basis of this unique synaptic function have been extremely successful in improving the design of artificial learning networks. Conditioning-specific changes of particular proteins have been related to m-RNA changes during the last year. The functional roles of these proteins as well as their identities are now being explored. One, for example, has been linked to GTP-binding protein signal transduction. Another may have more importance for cell structure. These learning-induced changes of protein availability may represent an important step for consolidating, i.e., making more permanent the physiologic memory trace which could have expression in conditioning-specific structural changes of *Hermissenda* neurons. These latter changes, as studies with the formation of *Hermissenda* associations, involve an apparent reorganization of the cell's terminal branches on which synaptic interactions occur.

The experimental psychology program of the Section uses associative learning paradigms to produce persistent behavioral changes in the nudibranch mollusc *Hermissenda crassicornis* as well as vertebrate species such as rabbits and rats. Quantitative assessments are made of the animals' responses to the conditioned and unconditioned stimuli before and after classical conditioning paradigms. These assessments include precise dissection of generalized behavioral transformations into modification of individual muscular components of the behaviors. A full range of psychological manipulations have been used to clearly establish the sensitivity of the learning behavior to the exact temporal relationship of the stimuli which are associated during acquisition of the learning. Also of interest to the psychologists is the close linkage of the learning behavior to the specific stimuli associated and discriminative functions involving those stimuli not associated. Recently this aspect of the program has been extended to include more cognitively oriented learning tasks involving spatial mapping of an organism's environment.

The Section's neurophysiology program is concerned first with the definition of those neural systems relevant to the learning capability. Multiple intracellular recordings from pre- and post-synaptic neurons have been employed within the visual, vestibular and chemosensory pathways of *Hermissenda* to establish a working knowledge of the critical neural systems and to describe how information flows in a stepwise fashion beginning with sensory cells at the output. A similar approach is being taken with the rabbit hippocampus and more recently the cerebellum and critical afferent and efferent pathways within these structures. Neurophysiological correlates are then obtained for conditioned (as well as a variety of control) animals. These neurophysiological correlates are recorded in intact animals, isolated nervous systems, and isolated neuronal membranes. Based on such correlates electrophysiological sequences are constructed to trace the transformation of the information in electrical terms of the neural system.

The Section's biophysics program measures persistent modification of specific ionic channels during and following the learning. In the past, a two microelectrode voltage clamp was employed to characterize genetically specified membrane currents within identified neurons which were demonstrated to play a causal role in the acquisition and retention of associative learning. More recently the patch-clamp technique has been used in both the cell-attached and "inside-out" configurations to determine which subcellular biochemical processes (e.g.,  $\text{Ca}^{2+}$ -dependent phosphorylation) are critical for regulating those ionic channels which change during learning. All of these biophysical approaches have also been applied to unequivocally demonstrate that it is in fact persistent modification of specific ionic channels which encode a learned association for later recall.

The biochemistry research of the Section seeks to uncover the molecular basis for the persistent ionic channel modifications shown to underlie associative learning (both in *Hermisenda* and the rabbit). A variety of biochemical and molecular biological methods are being brought to bear for this purpose. Microgel analysis of phosphorylation of individual neuronal proteins, for example, has revealed that  $\text{Ca}^{2+}$ -dependent phosphorylation of a specific low-molecular weight protein changes within certain neurons of conditioned animals but not those exposed to control paradigms. Exposure of neurons to prolonged depolarization, which simulated the integrated visual-vestibular network effects on identified neurons during conditioning, is also followed by long-lasting phosphorylation differences for particular low molecular weight proteins. More recently, learning-induced differences in the amount and/or the synthesis of these and other proteins have been detected. Recently, it has been possible to isolate specific proteins and inject them into specific neurons, thereby reproducing the actual learning effects on membrane channels. Furthermore, a number of intracellular manipulations have provided support for the hypothesis that learning-induced modification of ionic channels involves  $\text{Ca}^{2+}$ -calmodulin-dependent and  $\text{Ca}^{2+}$ -lipid-dependent phosphorylation. Such manipulations include iontophoretic injection of  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase, inositol triphosphate or PKC, or preincubation with PKC activators such as phorbol esters or OAG. Modern molecular biological techniques are also now making available for the Section's use monoclonal antibodies to enzymes implicated in the learning process. Other antibodies may also prove helpful for our reconstruction of the biochemical and associated biophysical sequences which make biological records of memory possible. Recombinant techniques may be particularly helpful in identifying specific proteins whose synthesis is modified as a result of the recently observed conditioning-specific increase in m-RNA turnover.

The cellular anatomy aspects of the Section's programs contributes in several ways to the various levels of inquiry into the learning process already mentioned. Ultrastructural measurements of the cells and their synaptic interaction has provided further definition of the relevant neural systems. Activity-dependent uptake of radioactive labels within these systems has been monitored by autoradiographic methods. Morphometric techniques together with serial sectioning and computerized reconstruction, have recently uncovered structural manifestations of the biophysical and biochemical changes already shown for neurons within conditioned but not control animals. Differential absorption spectrophotometry allows intracellular localization of fluctuations of cytosolic  $\text{Ca}^{2+}$ -dependent modulation of the channels during learning.



Finally, the developmental biologists within the Section have established laboratory strains of *Hermissenda*. Such strains permit assessment of how genetic and environmental factors may interact to determine individual differences in the ability of the animals to undergo associative learning. Furthermore, critical biochemical steps have now been identified which may serve potent cell-transforming functions in learning, developmental, and oncogenic contexts.

Perhaps most important in all of these efforts is the accumulated evidence that a remarkable similarity exists between means of encoding learned associations in the snail, rabbit, and now the rat. The same learning-induced reduction of well-characterized K<sup>+</sup> currents has been found to provide such encoding in *Hermissenda* as it does within identified neurons of rabbit hippocampal slices. Similar regulation of these channels appears to occur at the molecular level for both the mollusc, rabbit, and the rat. Such parallel mechanisms may ultimately provide the basis for clinical intervention and thereby the amelioration of pathologic symptoms.

### Section on Myelin and Brain Development

Research in the Section on Myelin and Brain Development is divided into two related areas falling under projects entitled "Glycoproteins of Myelin in Development and Disease" and "Antibodies to Glycoconjugates in Neurological Diseases", respectively. The first project, which has been in existence for many years, emphasizes the myelin-associated glycoprotein (MAG), although other myelin proteins are also studied with the ultimate objectives of understanding molecular mechanisms of myelin formation and breakdown. The second project is an offshoot of the first and deals with the occurrence and pathogenic significance of antibodies to MAG, other glycoproteins and glycolipids in neurological diseases, especially peripheral neuropathies.

#### 1. Structure and function of the myelin-associated glycoprotein (MAG).

Previous immunocytochemical studies had shown that MAG is selectively localized in periaxonal Schwann cell and oligodendroglial membranes of myelin sheaths suggesting that this glycoprotein functions in the formation and maintenance of the junction between these myelin-forming cells and the axolemma. MAG is also present in Schmidt-Lanterman incisures, lateral loops and the outer mesaxons of PNS myelin sheaths and is believed to play a role in maintaining the spacing of adjacent Schwann cell membranes in these structures where extracellular membrane surfaces are separated by 12-14 nm and there is always cytoplasm at the inner membrane surface. Collaborative immunocytochemical experiments at the electron microscope level with Dr. Bruce Trapp of Johns Hopkins University have now revealed two striking differences between the localizations of MAG in the CNS and PNS. First, unlike the PNS, MAG is not present in paranodal regions, incisures or outer mesaxons in the CNS, suggesting a more restricted function for MAG in oligodendrocyte-axon interactions in the CNS. Second, during active myelination, oligodendrocytes contain numerous multivesicular bodies (MVBs) that are enriched in MAG. MVBs are known to be endocytosed structures in other cells, and this raises the possibility that these MAG enriched bodies are carrying a signal from the periaxonal regions back to the oligodendrocyte cell bodies.

The myelin-associated glycoprotein has a single membrane spanning domain separating its C-terminal tail from a heavily glycosylated N-terminus that is composed of 5 domains related in amino acid sequence to members of the immunoglobulin superfamily such as neural cell adhesion molecule (N-CAM). These



extracellular immunoglobulin-like domains must mediate the function of MAG in glia-axon interactions, possibly by specifically interacting with another member of the superfamily on the axolemma. Homophilic MAG binding may be involved in the interactions of adjacent Schwann cell membranes in incisures, lateral loops and mesaxons. MAG has 8 extracellular sites for N-linked glycosylation, and detailed investigation of the oligosaccharides now underway indicates substantial heterogeneity in size and charge. Preliminary results also indicate that MAG is abnormally glycosylated in the dysmyelinating quaking mutant. Differences in oligosaccharide structure may modulate the functioning of MAG in membrane-membrane interactions.

MAG occurs in two forms differing in the lengths of their C-terminal domains and arising by alternative splicing of the primary mRNA transcript. The two forms are developmentally regulated in brain, with the larger form being most prominent at the time of active myelination, whereas nearly all the MAG in peripheral nerve is of the shorter form at all ages. Both PNS and CNS MAG are phosphorylated by incubation of tissue slices with inorganic [ $^{32}$ P]phosphate or incubation of isolated myelin with radioactive ATP. PNS MAG is more heavily phosphorylated than CNS MAG, a surprising result in view of the fact that the shorter form of MAG in the PNS has substantially fewer potential phosphorylation sites than the longer MAG molecules in the CNS. Regulation of expression of the two forms of MAG as well as phosphorylation of the cytoplasmic domains may modulate interactions with cytoskeletal components. Overall, the MAG molecule seems well suited to mediate interactions between intracellular cytoskeletal elements and adjacent extracellular membrane surfaces. In this manner, MAG could play a key role in the chemo-mechanical forces involved in generating the spiraled myelin sheaths.

An investigation of MAG in cultured oligodendrocytes and Schwann cells is under way with the ultimate objectives of investigating factors that control its expression and probing its function as an adhesion molecule. Cultured oligodendrocytes constitutively express MAG and other myelin proteins as demonstrated by immunostaining, Western blotting, and radioimmunoassay. Schwann cells cultured from young rats do not constitutively express MAG, but can be induced to express a small amount by elevation of cAMP levels with forskolin. The levels of MAG in the oligodendrocytes and forskolin treated Schwann cells are at the lower limits of detection, making detailed investigation of MAG in these cells difficult. However, an immortalized line of Schwann cells (S-16) has been generated by multiple passaging that expresses a remarkably high level of MAG, similar to that in adult sciatic nerve. This cell line does not express detectable levels of PO glycoprotein or myelin basic protein. Most of the MAG expressed by the continuous line of Schwann cells is on the surface, and it is hoped that these cells will be useful for studying MAG function in cell-cell interactions.

## 2. MAG in multiple sclerosis (MS)

Previous immunocytochemical and quantitative biochemical studies had demonstrated a greater loss of MAG than other myelin proteins at the periphery of MS plaques, suggesting an important role for MAG in the molecular pathology of this disease. Using a very sensitive solid phase radioimmunoassay, small, but significant elevations of antibodies to MAG were detected in the cerebrospinal fluids (CSF) of MS patients. It does not seem likely that this weak immune response to MAG could account for its selective early loss in MS plaques, and our favored hypothesis to explain the latter phenomenon involves the high susceptibility of MAG to a myelin-associated,  $\text{Ca}^{++}$ -activated, neutral protease as discussed in more detail in last year's report. The slightly elevated level of CSF antibodies to MAG may be

secondary to the principal cause of demyelination in MS, but it could play a role in progression of the disease.

### 3. Antibodies to glycolipids in peripheral neuropathies.

This area of investigation began with the demonstration of monoclonal anti-MAG antibodies in patients with mixed motor-sensory polyneuropathies occurring in association with IgM gammopathy. It was subsequently demonstrated that these anti-MAG antibodies cross reacted with the newly discovered sphingoglycolipid, sulfate-3-glucuronyl paragloboside (SGPG). Further studies showed that monoclonal IgM antibodies in other neuropathy patients did not react with MAG and SGPG but that they did react with various ganglioside antigens. Overall about 80% of patients with neuropathy occurring in association with IgM paraproteinemia have a monoclonal antibody that reacts with SGPG or ganglioside antigens, suggesting that glycolipids may be important target antigens in this type of neuropathy. Also, high titers of polyclonal antibodies to gangliosides were detected in about 15 to 20% of patients with acute demyelinating inflammatory polyneuropathy, i.e. Guillain-Barre syndrome, and the titers fell concurrently with clinical improvement.

In this fiscal year, our research in this area has emphasized antibodies to GM1 ganglioside in patients with motor nerve disorders. Three patients with multifocal motor neuropathy were found to have high titers of polyclonal antibodies to GM1 ganglioside, and the titers decreased in parallel with clinical improvement in two of the patients that were treated by immunosuppression. The fine specificities of the antibodies differed in the three patients as revealed by identification of different cross reacting gangliosides. Since GM1 was the only common antigen in all three patients, the results suggest that it could be the pathogenically significant target antigen. These findings and other results reveal a strong correlation between the occurrence of antibodies to GM1 ganglioside and motor nerve disorders, both in the presence and absence of monoclonal IgM gammopathy.

## Membrane Biochemistry Section

The Membrane Biochemistry Section is actively investigating the structure, biosynthesis and regulation of cell membrane components involved in various recognition phenomena and in cellular signaling. These include complex glycoconjugates such as gangliosides and the receptor-coupled adenylate cyclase and phospholipase C systems which mediate the cellular responses to various hormones, neurotransmitters and growth factors as well as pathological toxins and viruses. Most of our studies involve the use of cultured cell lines which express these components and respond to physiological and environmental signals.

### 1. Role of Ganglioside Lipid Moiety in Action of Cholera Toxin.

In an extensive series of experiments, we had previously established that ganglioside GM<sub>1</sub> is the only endogenous cell surface receptor for cholera toxin, the active agent in the disease cholera. The toxin intoxicates target cells by ADP-ribosylation of the stimulatory G protein, G<sub>s</sub>, of adenylate cyclase which thus results in a persistent activation of the cyclase. Although glycosphingolipids and glycoproteins sometimes have similar carbohydrate determinants, no such GM<sub>1</sub>-type "ganglioproteins" have yet to be identified. In order to explore the possibility that such glycoconjugates might also serve as receptors for cholera toxin, we developed a method to attach the oligosaccharide of GM<sub>1</sub> to proteins on the surface of viable cells. We released the oligosaccharide from GM<sub>1</sub> by ozonolysis, reductively aminated it and coupled it to a

heterobifunctional crosslinker. The resulting derivative covalently attaches to free sulfhydryl groups including those on proteins. We incubated the derivative with rat glioma cells which are deficient in  $G_{M1}$ , bind only traces of cholera toxin and are poorly responsive to the toxin. C6 cells exposed to the crosslinking derivative exhibited an increase in iodinated cholera toxin binding. Using a sensitive "Western" blotting technique, we identified the newly created toxin binding sites as modified proteins containing  $G_{M1}$ -oligosaccharide. Although cells treated with the crosslinking derivative bound large amounts of toxin, they remained as unresponsive to the toxin as control cells in terms of cyclic AMP accumulation and adenylate cyclase activation. This was in sharp contrast to  $G_{M1}$ -treated cells which exhibit an enhanced response to cholera toxin. Thus, these newly created cell surface "neoganglioproteins" behave as nonfunctional receptors for cholera toxin.

Our results indicate that the lipid moiety of  $G_{M1}$  is important for the action of cholera toxin. In addition, they suggest that  $G_{M1}$  does not act as a passive "acceptor" for cholera toxin binding but plays a more active role in cholera toxin action. Our results also raise an interesting paradox about the mechanism of action of cholera toxin compared to other bacterial toxins which ADP-ribosylate the G proteins of adenylate cyclase. The *E. coli* heat-labile enterotoxin has considerable structure homology with cholera toxin and also activates adenylate cyclase by ADP-ribosylation of  $G_s$ . Although  $G_{M1}$  can serve as a functional receptor for the heat-labile toxin, there is considerable evidence that the toxin can bind to intestinal brush border glycoproteins of certain species and that such glycoproteins can serve as functional receptors. Pertussis toxin which ADP-ribosylates the inhibitory G protein,  $G_i$ , of adenylate cyclase also is an oligomeric toxin. It consists of an active A protomer and a binding B oligomer. The latter appears to bind to cell surface sialoglycoproteins and not gangliosides and, in contrast to cholera and heat-labile toxin, readily intoxicates rat glioma C6 cells. Thus, bacterial toxins with similar intracellular targets (G proteins) are able to utilize different classes of cell surface receptors. Whether they use different mechanisms of entry into the cell such as direct membrane penetration or endocytosis remains to be established and is being investigated by us. In this regard, it is believed that the A protomer of cholera toxin directly penetrates the plasma membrane.

## 2. Receptors for Neurotransmitters and Neuropeptides

We are continuing to make progress on identification and characterization of the receptors for important neurotransmitters and neuropeptides. These include  $\beta$ -adrenergic,  $D_1$  dopaminergic, muscarinic and neuropeptide Y receptors. Using our recently developed procedures for solubilizing and purifying the  $D_1$  receptor from rat striatum, we have succeeded in obtaining a 5000 - to 8000-fold purified receptor preparation. This is very close to the theoretical value of 14 nmol of bound ligand per mg protein for a pure protein of 70 kDaltons and one binding site. When the purified receptor was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining, two major bands of 70 and 50 kDaltons were observed, which we believe may represent glycosylated and deglycosylated forms of the receptor. A  $D_1$  receptor-specific photoaffinity radioligand labeled both of these proteins in striatal membranes and in the purified preparation. The purified receptor displayed the same pharmacological characteristics as the membrane receptor for stereoisomers of  $D_1$  dopamine agonists and antagonists. To our knowledge, this represents the most highly purified  $D_1$  dopamine receptors reported to date. We are now scaling up the procedure in order to obtain sufficient receptor protein for amino acid sequencing, the raising of antibodies, and ultimately the cloning of the receptor gene.



Although the existence of D<sub>1</sub> dopamine receptors is well-established, progress in understanding their regulation has been hampered by lack of an appropriate model cultured cell line. We screened a number of neurotumor cell lines and found that SK-N-MC cells possessed specific and functional D<sub>1</sub> receptors. When SK-N-MC cells were exposed to dopamine, they accumulated cyclic AMP in a time and dose dependent manner. Intracellular cyclic AMP levels increased over 100-fold and half-maximal increases were observed at 2  $\mu$ M dopamine ( $K_{act}$ ). This response was mimicked by a D<sub>1</sub> agonist, (R) SKF 38393, but not its inactive S-enantiomer, and was inhibited by a D<sub>1</sub> antagonist, (R)SCH 23390, whereas the S-enantiomer was over 50-fold less potent. Membranes prepared from SK-N-MC cells bound the D<sub>1</sub> ligand [<sup>125</sup>I]SCH 23982 with a single, high affinity state. Binding was appropriately displaced by dopamine and the R-enantiomers of SKF 38393 and SCH 23390, but less potently by the corresponding S-enantiomers. Agonist competition curves indicated the existence of two affinity states with around 40% of the receptors in a high affinity, guanine nucleotide-sensitive state, a proportion much higher than found in striatum (only 6%). The D<sub>1</sub> receptor-specific photaffinity probe labeled a 72 kDalton protein in SK-N-MC cell membranes, similar to the protein labeled in rat striatal membranes. Thus, SK-N-MC human neuroblastoma cells contain D<sub>1</sub> dopamine receptors which are pharmacologically similar to those found in mammalian striatum, but which are more tightly coupled to adenylate cyclase. SK-N-MC cells may be a useful model to investigate the properties and regulation of D-1 dopamine receptors. We are now examining the effects of exposure of the cells to dopamine as well as other drugs on the levels of D<sub>1</sub> receptors and their coupling to adenylate cyclase. Based on our earlier research on regulation of  $\beta$ -adrenergic receptors, we know that agonists induce receptor desensitization and down-regulation.

Neuropeptide Y (NPY) is the most abundant neuropeptide in the mammalian nervous system. It is wide spread, is found in both central and peripheral neurons, is often colocalized with catecholamines, and has several physiological effects, including appetite stimulation and vasoconstriction. Little is known about its mode of action or its receptors. We have previously reported that in SK-N-MC cells, NPY inhibits adenylate cyclase and this inhibition is blocked by pertussis toxin. We have now been able to identify NPY receptors and G<sub>i</sub> in these cells. SK-N-MC cells bound [<sup>3</sup>H]-NPY in a time and dose dependent manner. The bound radioligand was displaced by unlabeled NPY. From both kinetic and equilibrium binding data, a  $K_D$  of 2 nM and a  $B_{max}$  of 100,000 receptors per cell were determined. This compares favorably with the  $K_{act}$  of 0.5 nM for NPY inhibition of adenylate cyclase activity in these cells. Pertussis toxin catalyzed the ADP-ribosylation of a 41 kDalton protein in SK-N-MC membranes, which corresponds to the inhibitory G protein, G<sub>i</sub>. Thus, SK-N-MC cells contain all the necessary components for characterizing NPY receptors and their regulation.

At least five different subtypes of muscarinic acetylcholine receptors have been cloned to date. Our initial studies showed that muscarinic agonists only stimulated phosphoinositide turnover in SK-N-SH human neuroblastoma cells with m3 receptors and inhibited adenylate cyclase in NG108-15 mouse neuroblastoma x rat glioma cells with m4 receptors. Although a number of muscarinic antagonists exhibited similar binding properties for both receptor subtypes, some agonists exhibited higher affinity for NG108-15 receptors than for SK-N-SH receptors. The possibility that certain muscarinic agonists may be selective for receptors coupled to inhibition of adenylate cyclase vs. those coupled to phosphoinositide turnover was explored. A number of derivatives of oxotremorine were synthesized based on a functional congener approach. Each compound was screened both for its ability to compete

with [ $^3\text{H}$ ]-N-methylscopolamine binding to cell membranes and its effect on phosphoinositide turnover and production of cyclic AMP. Compound BM 5, which is a postsynaptic partial agonist and a presynaptic antagonist, was found to inhibit adenylate cyclase in NG108-15 cells and in rat heart (m2 receptors) but only partially as compared to the full agonist, oxotremorine-M. In contrast, BM5 did not stimulate phosphoinositide turnover in SK-N-SH cells or in Chinese hamster ovary cells transfected with and expressing m1 receptors. In these latter two cell lines, BM5 behaved as a full antagonist by inhibiting oxotremorine-M stimulation. Two additional derivatives were found to have similar characteristics to BM5 but with higher ratios of activity to affinity. These studies raise the possibility that post-synaptic muscarinic receptors are coupled to adenylate cyclase and pre-synaptic receptors to phospholipase C. The ability to selectively stimulate post-synaptic receptors may be clinically useful for treating neurological diseases with a cholinergic deficit such as Alzheimer's disease.

### 3. Differential Activation of G Proteins in Cells and Membranes

It is well-established that many receptors for hormones and neurotransmitters are coupled to their effector systems through guanine nucleotide binding proteins (G proteins). Thus, stimulatory receptors activate adenylate cyclase through  $G_s$  and inhibitory receptors inhibit adenylate cyclase through  $G_i$ . These G proteins are heterotrimers with common  $\beta\gamma$  and distinct  $\alpha$  subunits. The latter can be ADP-ribosylated by bacterial toxins,  $\alpha_s$  by cholera and  $\alpha_i$  by pertussis toxin. Whereas activated  $G_s$  is more readily ADP-ribosylated by cholera toxin, inactive  $G_i$  is ADP-ribosylated by pertussis toxin. It has been proposed that activation of G proteins involves exchange of bound GDP for GTP on the  $\alpha$  subunit which then dissociates from the  $\beta\gamma$  subunits. In the case of  $G_s$ , the GTP-occupied  $\alpha_s$  then activates the catalytic component of adenylate cyclase until the bound GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the  $\alpha$  subunit. The latter then reassociates with the  $\beta\gamma$  subunits to complete the cycle. The situation with  $G_i$  is more complex as  $\alpha_i$  only weakly inhibits the catalytic component; the  $\beta\gamma$  subunits, however, bind to  $\alpha_s$  and keep it in its inactive state. As  $G_i$  is found in large excess to  $G_s$ , it appears that the latter mechanism is the major one for inhibition. Normally, the agonist-occupied receptor mediates this activation of G proteins, but hydrolysis-resistant analogues of GTP and fluoroaluminate also can persistently activate G proteins as the GTPase "turnoff" is no longer operating.

It has been known since the early work of Sutherland and Rall that sodium fluoride (actually the fluoroaluminate ion) stimulates adenylate cyclase in membranes. Fluoroaluminate, however, fails to stimulate the cyclase in intact cells and actually inhibits agonist stimulation. Using pertussis and cholera toxin as probes we were able to clearly establish that the effects of fluoroaluminate on intact cells are due to its potent activation of  $G_i$ . Fluoroaluminate inhibited ADP-ribosylation of  $G_i$  in both intact cells and isolated membranes, which indicated that  $G_i$  was being activated and dissociated to  $\alpha_i$  and  $\beta\gamma$  subunits. In addition, fluoroaluminate inhibited agonist-enhanced ADP-ribosylation of  $G_s$  by cholera toxin in both intact cells and membranes, which indicated that the activation and dissociation of  $G_s$  was being inhibited. We believe that the latter inhibition is due to the  $\beta\gamma$  subunits from  $G_i$  complexing and deactivating it. This is the first time that the proposed model for inhibition of adenylate cyclase by dissociation of  $G_i$  has been demonstrated in intact cells. All previous studies involved the purified subunits and membrane preparations. We are now trying to resolve why fluoroaluminate can activate  $G_i$  in intact cells but not  $G_s$ . In this regard, we observed that both were activated in cells made permeable with digitonin. In the latter cells, fluoroaluminate by itself



stimulated adenylate cyclase at low concentrations and inhibited agonist-stimulated activity at higher concentrations. This is consistent with the model as  $G_i$  is in excess of  $G_s$ . It also indicated that a slight perturbation of the plasma membrane allows fluoroaluminate access to  $G_s$ . We also plan to extend our studies to inhibitory receptors and their ligands which are the normal activators of  $G_i$  such as NPY and its receptor.

## **SECTION OF RECEPTOR BIOCHEMISTRY AND MOLECULAR BIOLOGY**

This has been a very productive year for the Section of Receptor Biochemistry and Molecular Biology, with progress being made in all areas of research. Several milestones have been reached this year with a number of significant findings and accomplishments that will have impact on this field.

Key areas of investigation include 1) the molecular biology of neurotransmitter receptors; 2) the expression and site directed mutagenesis of neurotransmitter receptor genes; and 3) large scale DNA sequencing of neurotransmitter receptor genes and human chromosomal regions to which neurological disorders with a genetic basis have been mapped.

The neurotransmitter receptors under study in the Section include muscarinic cholinergic, alpha and beta-adrenergic, nicotinic and GABA/benzodiazepine, octopamine and dopamine receptors. Accomplishments pertaining to the study of these receptors include the cloning, sequencing and expression of the human alpha2A-adrenergic receptor, the *Drosophila* octopamine and muscarinic acetylcholine receptor, the rat M1 and M3 muscarinic acetylcholine receptors, the human alpha1-adrenergic receptor and a locust nicotine acetylcholine receptor.

We have utilized the DNA and deduced amino acid sequences from the above studies to extend our work on evolution of multi-gene families.

The cloning and sequencing of genes encoding neurotransmitter receptors have revealed the existence of several gene families containing related receptor proteins. One of the families contains receptors that mediate the actions of hormones and neurotransmitters by guanine nucleotide regulatory proteins. Included in this family are the adrenergic, muscarinic cholinergic, substance k, octopamine, serotonin and yeast mating factor receptors as well as the opsins and bacteriorhodopsins. Recent data from the cloning and sequencing of several members of this gene family have indicated that these proteins share considerable homology at both the gene and protein levels. When the deduced protein sequences are analyzed for secondary structure, each protein has been shown to contain seven stretches of hydrophobic amino acids that are believed to represent seven membrane spanning domains. Expression of the genes encoding neurotransmitter receptors in various systems allows for the detailed examination of receptor structure and function. We were the first to describe the production of stable cell lines expressing human alpha2-adrenergic receptors and illustrate the utility of such lines in the biochemical and pharmacological characterization of receptor proteins.

One of the cell lines obtained was utilized to study alpha2-adrenergic mechanisms of signal transduction. Permanent expression of the alpha2-receptor was achieved by transfecting Chinese hamster ovary (CHO) cells which lack adrenergic receptors with a 1.5-kilobase NcoI-HindIII fragment of the genomic clone containing the coding region of the gene. The alpha2-receptor expressed in CHO cells displayed

pharmacology characteristic of an  $\alpha_2A$ -receptor subtype with a high affinity for yohimbine ( $K_i = 1 \text{ nM}$ ) and a low affinity for prazosin ( $K_i = 10,000 \text{ nM}$ ). The role of the  $\alpha_2$ -receptor in modulating intracellular cyclic AMP concentrations was investigated in three transfected cell lines expressing 50, 200, and 1200 fmol of receptor/mg membrane protein. At low concentrations (1-100 nM), (-)-epinephrine attenuated forskolin-stimulated cyclic AMP accumulation by up to 60% in a receptor density-dependent manner. At epinephrine concentrations above 100 nM, cyclic AMP levels were increased up to 140% of the forskolin-stimulated level. Pertussis toxin pretreatment of cells eliminated  $\alpha_2$ -receptor-mediated attenuation of forskolin-stimulated cyclic AMP levels and enhanced the receptor density-dependent potentiation of forskolin-stimulated cyclic AMP concentrations from 3 to 8-fold. Potentiation of forskolin-stimulated cyclic AMP levels was also elicited by the  $\alpha_2$ -adrenergic agonists, UK-14304 and para-aminoclonidine, and blocked by the  $\alpha_2$ -adrenergic antagonist yohimbine, but not by the  $\alpha_1$ -adrenergic antagonist prazosin or the  $\beta$ -adrenergic antagonist propranolol.  $\alpha_2$ -receptor-mediated potentiation of forskolin-stimulated adenylate cyclase activity is apparently not due to activation of phospholipase C, as epinephrine had no effect on phosphoinositide hydrolysis in transfected cells, or to  $\text{Na}^+/\text{H}^+$  exchange, as the potentiation was unaffected by ethylisopropylamiloride at concentrations up to 100  $\mu\text{M}$ . The nonselective phospholipase A2 inhibitor quinacrine antagonized the  $\alpha_2$ -receptor-mediated stimulation of cyclic AMP production in pertussis toxin-treated cells in a dose-dependent manner. In cells treated with quinacrine in the absence of pertussis toxin, epinephrine produced up to a 90% inhibition of forskolin-stimulated cyclic AMP concentrations in a dose-dependent manner with no increases in cyclic AMP production. These data suggest that the human  $\alpha_2$ -adrenergic receptor in CHO cells may simultaneously couple to more than one effector including a pertussis toxin-sensitive attenuation of adenylate cyclase and a pertussis toxin-insensitive pathway that results in potentiation of intracellular cyclic AMP levels.

Our previous studies have characterized M1 muscarinic acetylcholine receptors permanently expressed in cultured cells. M1 and M3 receptors display significant structural homology and thus it was of interest to determine the degree of functional homology between these receptor subtypes. A rat M3 muscarinic receptor was isolated from a rat genomic library using a probe derived from the amino terminal fragment of a rat M2 muscarinic receptor. The identity of the receptor was determined by hybridization with a rat M3 muscarinic receptor specific oligonucleotide of 49 bases, by restriction endonuclease mapping and by partial sequence analysis. A 1.8kb Acc1-TthIII1 fragment containing the entire coding region of the rat M3 muscarinic receptor was subcloned into the Sma1 and Sac1 sites of the expression vector, pSVL, after blunt ending the 5'-TthIII1 end with S1 nuclease. Chinese hamster ovary-K1 (CHO) cells were cotransfected with the expression vectors pSVL containing M3 muscarinic receptor and pMSVneo containing the gene for neomycin resistance using the calcium phosphate precipitation technique. The biochemical and pharmacological properties of the rat M3 muscarinic receptor were fully characterized by ligand binding experiments using agonists and antagonists, by measurement of agonist stimulated changes in intracellular cyclic AMP accumulation and phosphoinositide turnover and by affinity labeling with [3H]-propylbenzilycholine mustard. The rat M3 muscarinic receptor displayed saturable, high affinity binding of [3H]-quinuclidinyl benzilate (QNB) with a  $K_d$  of 16 pM and a rank order of potency of antagonists of  $\text{QNB} > \text{atropine} > \text{pirenzepine} > \text{AF-DX 116}$ , similar to an M1 muscarinic receptor. The rat M3 and the rat M1 muscarinic receptors mediated agonist-induced increases in phosphatidylinositol metabolism. In addition, the M1 receptor stimulated cAMP production, an effect not seen with the rat M3 muscarinic receptor. Results from propylbenzilycholine mustard labeling

showed the rat M3 and the rat M1 muscarinic receptors migrated on SDS-PAGE gels with apparent molecular masses of 94,000 and 84,000 daltons, respectively, which is consistent with the known differences in the primary structure of these receptors.

Using DNA probes from mammalian adrenergic and muscarinic acetylcholine receptors, we screened a *Drosophila* cDNA library for cross-hybridizing genes.

A complementary DNA (cDNA) for the *Drosophila* octopamine1 receptor was isolated using a probe derived from a human beta2-adrenergic receptor cDNA. This clone encodes a protein of 601 amino acids and displays homology with all adrenergic receptors. A 2.2 kb DNA fragment of this cDNA clone was inserted into the plasmid expression vector, pSVL, and co-transfected with pMSVneo into CHO-K1 cells. Cells expressing the *Drosophila* gene were selected by growth in selective medium. The *Drosophila* receptor bound [3H]-yohimbine with a Kd of 6 nM. Agonists displayed a rank order of potency for inhibition of radioligand binding of synephrine > clonidine = octopamine > serotonin > epinephrine >> isoproterenol and dopamine; antagonists displayed a rank order of potency of chlorpromazine > mianserin > phentolamine > cyproheptadine > metoclopramide > propranolol. The rank order of potency of these agents in inhibiting [3H]-yohimbine binding suggested that we had cloned an octopamine1 receptor. Addition of octopamine to transfected cells resulted in a dose-dependent attenuation of forskolin stimulated cAMP levels. the octopamine1 receptor gene is localized on the right arm of *Drosophila* chromosome 3 and is preferentially expressed in neuronal tissue. Sequence and pharmacological comparisons indicate that the octopamine receptor is unique but closely related to mammalian adrenergic receptors, perhaps as an evolutionary precursor.

Two cDNA clones (3.7 kb and 4.8 kb) encoding a *Drosophila* muscarinic acetylcholine receptor were isolated from a *Drosophila* head cDNA library and characterized by automated DNA sequence analysis. The *Drosophila* muscarinic receptor contains 788 amino acids with a calculated Mr of 84,807 and displays greater than 60% homology with mammalian muscarinic receptors. The muscarinic receptor maps to the tip of the right arm of the second chromosome of the *Drosophila* genome. Together these findings suggest that the origins of the G-protein linked receptor family can be traced back several hundred million years in evolution.

In order to examine the functional significance of single amino acids in more detail, we continued our work on site-directed mutagenesis of neurotransmitter receptors. Using site-directed mutagenesis of the human beta2-adrenergic receptor and continuous expression in B-82 cells, the role of 3 conserved cysteines in the third extracellular domain in receptor function was examined. Substitution of cysteine 285, in the sixth transmembrane domain of the receptor, produced a mutant receptor with normal ligand-binding properties but a significantly attenuated ability to mediate stimulation of adenylate cyclase. Mutation of cysteine residues 190 and 191, in the third extracellular loop of the beta2 receptor, had qualitatively similar effects on ligand binding and isoproterenol-mediated stimulation of adenylate cyclase. Replacement of either of these residues with serine produced mutant receptors that displayed a marked loss in affinity for both beta-adrenergic agonists and antagonists. Replacement of both cysteine 190 and 191 with serine had an even greater effect on the ability of the receptor to bind ligands. Consistent with the loss of Ser190 and /or Ser191 mutant receptor affinity for agonists was a corresponding shift to the right in the dose-response curve for isoproterenol-induced increases in intracellular cyclic AMP concentrations in cells expressing the mutant receptors. These data implicate one of the conserved transmembrane cysteine residues in the



human beta2-adrenergic receptor in receptor activation by agonists and also suggest that conserved cysteine residues in an extracellular domain of the receptor may be involved in ligand binding.

Muscarinic acetylcholine receptors contain a region encompassing the second and third transmembrane domains which is rich in conserved aspartic acid (Asp) residues. To investigate the role of four conserved aspartic acids at positions 71, 99, 105 and 122 in muscarinic receptor function, point mutations in the rat M1 muscarinic receptor gene were made that converted each Asp to asparagine (Asn), and wild type or mutant genes were stably expressed in Chinese hamster ovary (CHO) cells that normally lack muscarinic receptors. Substitution of Asp71 or Asp122 with Asn produced mutant receptors that displayed high affinity for carbachol but decreased efficacy and potency, respectively, in agonist activation of phosphoinositide hydrolysis, suggesting that these residues may play important roles in receptor-effector coupling. Substitution of Asp99 or Asp105 with Asn produced marked decreases in ligand binding affinities and/or covalent incorporation of [3H]-propylbenzilycholine mustard, suggesting that these residues may be involved in receptor-ligand interactions.

As part of our ongoing sequencing projects, we have been developing sequencing strategies and technologies which will allow the sequencing of megabase regions of DNA in a highly efficient manner. Our use of three Applied Biosystems automated DNA sequencers allows us to generate up to 24 kilobases of sequence data per day. We have made a number of technical advances in our sequencing protocols in the past year including improvement of the phagemid template preparation procedure, automation of the generation of overlapping deletions with exonuclease III using a Biomek 1000 automated workstation, optimization of the preparation of single stranded DNA template for sequencing, automation of the DNA sequencing reactions using a Biomek 1000 workstation, and the development of computer software to facilitate the transfer of sequence data from the automated sequencers to other computers for assembly and analysis. We are currently working on optimizing and automating each of these steps. We have obtained cosmid clones from the q28 region of the human X chromosome, which contains several loci of neurological significance. Clones have also been isolated for the two major classes of neurotransmitter receptor genes, the nicotinic acetylcholine/GABA and muscarinic acetylcholine/adrenergic receptor families. In addition, collaborations have been initiated which will allow clones associated with several important neurological diseases, including neurofibromatosis I and Huntington's Disease, to be obtained and sequenced.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS02151-15

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Information Processing in Simple Nervous Systems

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.L. Alkon Medical Officer LMCN NINDS

Others: C. Collin, Visiting Fellow, LMCN, NINDS; T. Nelson, Staff Fellow, LMCN, NINDS; J. Cosgrove, Staff Fellow, LMCN, NINDS; J. Blaszyk, Visiting Fellow, LMCN, NINDS; B. Bank, Guest Worker, LMCN, NINDS; R. Etcheberrigaray, Visiting Fellow, LMCN, NINDS; L. Matzel, IRTA Fellow, LMCN, NINDS; B. Schreurs, Staff Fellow, LMCN, NINDS; M. Anderson Hughs Fellow, LMCN, NINDS; P. Huddie, Visiting Fellow, LMCN, NINDS; I. Lederhendler, Staff Fellow, LMCN, NINDS; D. McPhie, Special Volunteer, LMCN, NINDS; J.V. Sanchez-Andres, Guest Researcher, LMCN

## COOPERATING NITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543 (A. Kuzirian); California Institute of Technology (C. Chen); Medical Research Council, Canada (B. Bank)

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP, DIR, NINDS

## SECTION

Neural Systems Section

## INSTITUTE AND LOCATION

Park Building, Room 431 and Building 9, Room 1W12S, NINDS, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

10.0

## PROFESSIONAL:

9.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal objective of the program is to define molecular and biophysical mechanisms of learning and memory. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals of such research are to arrive at clinically meaningful interventions and to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principle frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than non-associative behavioral modifications (such as sensory adaptation, habituation, arousal, and sensitization). The biological basis of learning and memory is of interest at several levels of complexity: behavioral phenomena, neuronal systems, neuronal membranes and molecular transformations. To literally reconstruct the physiology involved (and to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusc *Hermisenda crassicornis* as well as "complex system" preparations such as rabbits and rats. The molluscan work thus far has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of identified single neurons it has proven possible to define biochemical pathways which regulate such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations have been shown in our program to record associative memory in the rabbit as were found in *Hermisenda*. Rabbit and now rat neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., protein kinase C) pathways which control membrane excitability have recently been demonstrated.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02784-01LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of the Structure and Function of Gonadotropins and their Receptors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                       |   |             |
|---------|-----------------------|---|-------------|
| PI:     | R. V. Rebois, Ph.D.   | Head, Unit on Receptor Structure and Function | LMCN, NINDS |
| OTHERS: | Y. Inoue, M.D.        | Visiting Fellow                               | LMCN, NINDS |
|         | V. J. B. Reddy, Ph.D. | Visiting Fellow                               | LMCN, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

## SECTION

Membrane Biochemistry Section

## INSTITUTE AND LOCATION

Park Building, Room 408, NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.2

## PROFESSIONAL:

2.2

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. The carbohydrate moieties of glycopeptide hormones are required for biological activity. The glycopeptide hormone, human chorionic gonadotropin (hCG) stimulates the gonadotropin responsive adenylate cyclase in MLTC-1 cells. We use hCG stimulation of adenylylase in MLTC-1 cells as a model to study the role of carbohydrates in the biological activity of glycopeptide hormones. Some modifications of the carbohydrate moieties do not decrease the biological activity of hCG, indicating that some parts of carbohydrate structure are not critical for biological activity of the hormone. 2. Hormone responsive adenylylase is regulated by at least two guanine nucleotide binding proteins (G proteins), one that stimulates adenylylase,  $G_s$ , and one that inhibits adenylylase,  $G_i$ . Hormones that stimulate adenylylase do so by activating  $G_s$ . Fluoroaluminate can activate  $G$  proteins. In cells, fluoroaluminate activates  $G_i$ , causing inhibition of hormone stimulated cAMP accumulation, but fluoroaluminate does not activate  $G_s$ . In membranes, fluoroaluminate activates  $G_i$ , inhibiting hormonal activation of adenylylase. Fluoroaluminate also activates  $G_s$  in membranes, causing adenylylase to be activated despite the simultaneous activation of  $G_i$  by fluoroaluminate. Only at high concentrations of fluoroaluminate is the influence of  $G_s$  on adenylylase overcome by activation of  $G_i$ . 3. Gonadotropin responsive adenylylase in MLTC-1 cells can be desensitized by hCG, or by activators of the  $Ca^{2+}$ /phospholipid dependent protein kinase (PKC). However, hCG-induced desensitization is not caused by activation of PKC. Similarity between the characteristics of hCG- and PKC-induced desensitization implicates protein phosphorylation in the mechanism of desensitization.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02366-11LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Hormone-Responsive Adenylate Cyclase

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                      |                                      |             |
|---------|----------------------|--------------------------------------|-------------|
| PI:     | P. H. Fishman, Ph.D. | Chief, Membrane Biochemistry Section | LMCN, NINDS |
| OTHERS: | J. Baumgold, Ph.D.   | Special Expert                       | LMCN, NINDS |
|         | A. Sidhu, Ph.D.      | Staff Fellow                         | LMCN, NINDS |
|         | E. A. Gordon, Ph.D.  | IRTA Fellow                          | LMCN, NINDS |
|         | R. Paek              | Special Volunteer                    | LMCN, NINDS |
|         | J. Votipka           | Special Volunteer                    | LMCN, NINDS |

## COOPERATING UNITS (if any)

Laboratory of Chemistry, NIDDK

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

## SECTION

Membrane Biochemistry Section

## INSTITUTE AND LOCATION

NINDS, NIH, Park Building, Room 411, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.8

## PROFESSIONAL:

2.3

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

[3H]-labeled neuropeptide Y (NPY) binds to SK-N-MC human neuroblastoma cells; binding is of high affinity and corresponds to 100,000 receptors per cell. The receptors are functional as NPY inhibits agonist-stimulated adenylate cyclase in these cells and inhibition is blocked by pertussis toxin. The toxin ADP-ribosylates a 41 kDalton protein in SK-N-MC membranes which is Gi, the inhibitory G protein of the adenylate cyclase system. Dopamine stimulates cyclic AMP production in human neuroblastoma SK-N-MC cells to levels greater than 100-fold over basal. The response is mimicked by dopamine D-1 selective agonists and blocked by antagonists. The D-1 specific antagonist [<sup>125</sup>I]SCH 23982 binds to cell membranes; binding is characterized by a single high affinity site and is competed for by the appropriate stereoisomers of D-1 antagonists and agonists. Up to 40% of the receptors exist in an agonist high affinity state which is sensitive to guanine nucleotides. This indicates tight coupling of the receptors to Gs, the stimulatory G protein of the cyclase and may explain the large response to dopamine in these cells. As this is the first demonstration both by binding and biological response of functional dopamine D-1 and NPY receptors in an established cell line, the SK-N-MC cell line will be a useful model for investigating the regulation and properties of human D-1 and NPY receptors. The dopamine D-1 receptor has been purified 5000- to 8000-fold from rat striatum in high yield. The purified receptor preparation contains two major proteins of 72 and 50 kDaltons, both of which are labeled by [<sup>125</sup>I]-MAB, a D-1 specific photoaffinity ligand. This represents the most highly purified D-1 receptor to date and will be useful for obtaining antibodies, sequence data and eventually the receptor gene. Compound BM5 has been reported to be an agonist at post-synaptic muscarinic receptors and an antagonist at pre-synaptic ones. BM5 behaves as an agonist for m1 and m3 receptors coupled to the inhibition of adenylate cyclase and as an antagonist for m2 and m4 receptors coupled to stimulation of the phosphoinositide-specific phospholipase C. Thus, post- and pre-synaptic muscarinic receptors may represent different subtypes coupled to different signal transduction systems. BM5 may be a prototype for selective muscarinic drugs.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01N501309-24 LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                    |                                      |             |
|---------|--------------------|--------------------------------------|-------------|
| PI:     | P.H. Fishman, Ph.D | Chief, Membrane Biochemistry Section | LMCN, NINDS |
| OTHERS: | T. Pacuska, Ph.D   | Visiting Scientist                   | LMCN, NINDS |
|         | R.M. Bradley       | Chemist                              | LMCN, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

## SECTION

Membrane Biochemistry Section

## INSTITUTE AND LOCATION

NINDS, NIH, Park Building, Room 411, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.8

## PROFESSIONAL:

2.3

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is well established that ganglioside  $G_{M1}$  is the cell surface receptor for cholera toxin. The oligosaccharide chain of  $G_{M1}$  is recognized by the B or binding subunits of the toxin whereas the A subunit of the toxin activates adenylate cyclase by ADP-ribosylation of the stimulatory G protein of the cyclase system. Less is known about the role(s) that the lipid moiety of  $G_{M1}$  plays in toxin action. We synthesized a derivative of  $G_{M1}$ -oligosaccharide that covalently attaches to free sulfhydryl groups including those of proteins. We tested the derivative on rat glioma C6 cells which are deficient in  $G_{M1}$ , bind only traces of the toxin and are poorly responsive to it. Exposure of C6 cells to the  $G_{M1}$ -oligosaccharide crosslinking derivative resulted in an increase in iodinated toxin binding which was dependent both on time of exposure to the derivative and its concentration. Prior exposure of the cells to dithiothreitol further enhanced the attachment of the derivative to the cell surface. The nature of these toxin binding sites was determined by a Western blotting technique and shown to be a wide spectrum of membrane proteins, some of which were trypsin resistant, although cells treated with the crosslinking derivative bound large amounts of toxin, they remained as unresponsive to the toxin as control cells in terms of cyclic AMP accumulation or adenylate cyclase activation. This was in sharp contrast to  $G_{M1}$ -treated cells which exhibited an enhanced response to cholera toxin. Thus, these newly created cell surface neoganglioproteins behave as nonfunctional receptors for cholera toxin. Our results suggest that the lipid moiety of  $G_{M1}$  is important for the action of cholera toxin.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02786-01LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibodies to Glycoconjugates in Neurological Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard H. Quarles, Ph.D. Section Chief LMCN, NINDS  
 Others: Genevieve Daune, Ph.D. Visiting Fellow LMCN, NINDS  
 Antonio Noronha, Ph.D. Staff Fellow LMCN, NINDS  
 Johanna Moller, M.D. Vis. Associate LMCN, NINDS  
 Hiroko Baba, M.D. Visiting Fellow LMCN, NINDS  
 Jeffrey Hammer Biologist LMCN, NINDS

## COOPERATING UNITS (if any)

Dept. Neurology, Johns Hopkins Univ., Balto., MD; Medical Neurology Branch, NINDS;

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

## SECTION

Section on Myelin and Brain Development

## INSTITUTE AND LOCATION

Park Building, Rm. 425, NINDS, NIH, Bethesda, MD 20205

## TOTAL MAN-YEARS:

2.1

## PROFESSIONAL:

1.9

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research on monoclonal and polyclonal antibodies to glycoconjugates in neurological diseases developed as part of our research on the myelin-associated glycoprotein (MAG), and earlier research in this area was included in our Section's ongoing Project No. Z01-NS 01808. It is now being assigned a separate project number since this aspect of our research has taken on an identity of its own. This area of investigation began with the demonstration of monoclonal anti-MAG antibodies in patients with mixed motor-sensory polyneuropathies occurring in association with IgM paraproteinemia. It was subsequently demonstrated that these anti-MAG antibodies cross reacted with the newly discovered sphingoglycolipid, sulfate-3-glucuronyl paragloboside (SGPG). Further studies showed that monoclonal IgM antibodies in other neuropathy patients did not react with MAG and SGPG, but that they did react with various ganglioside antigens. Overall about 80% of patients with neuropathy occurring in association with IgM paraproteinemia have a monoclonal antibody that reacts with SGPG or ganglioside antigens, suggesting that glycolipids may be important target antigens in this type of neuropathy. Also, high titers of polyclonal antibodies to gangliosides were detected in about 15 to 20% of patients with acute demyelinating inflammatory polyneuropathy, i.e. Guillain-Barre syndrome, and the titers fell concurrently with clinical improvement. In this fiscal year, our research in this area has emphasized antibodies to GM1 ganglioside in patients with motor nerve disorders. Three patients with multifocal motor neuropathy were found to have high titers of polyclonal antibodies to GM1 ganglioside, and the titers decreased in parallel with clinical improvement in two of the patients that were treated by immuno-suppression. The fine specificities of the antibodies differed in the three patients as revealed by identification of different cross reacting gangliosides. These findings and other results reveal a strong correlation between the occurrence of antibodies to GM1 ganglioside and motor nerve disorders. In addition, using a very sensitive solid phase radioimmunoassay, small, but significant elevations of antibodies to MAG were detected in the cerebrospinal fluids of multiple sclerosis (MS) patients. It is likely that this weak immune response to MAG is secondary to the principal cause of demyelination in MS, but it could play a role in progression of the disease.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS 01808-20 LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glycoproteins of Myelin in Development and Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard H. Quarles, Ph.D. Section Chief LMCN, NINDS

Others: David Kobiler, Vis. Scientist LMCN, NINDS;

Carl Lauter, Chemist, LMCN, NINDS

Antonio Noronha, Staff Fellow LMCN, NINDS;

Jeffrey Hammer, Biologist, LMCN, NINDS

Johanna Moller, Vis. Associate LMCN, NINDS;

Mary McLenigan, Chemist, LMCN, NINDS

Hiroko Baba, Vis. Fellow LMCN, NINDS

Shuichiro Goda, Vis. Fellow LMCN, NINDS

Zbigniew Bartoszewicz, Vis. Fellow LMCN, NINDS

## COOPERATING UNITS (if any)

Dept. Neurology, Johns Hopkins Univ., Balto., MD; Dept. Pediatrics, Washington Univ., St. Louis, MO.

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

## SECTION

Section on Myelin and Brain Development

## INSTITUTE AND LOCATION

Park Building, Rm. 425, NINDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

7.1

## PROFESSIONAL:

4.3

## OTHER:

2.8

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The myelin-associated glycoprotein (MAG) is localized in the periaxonal membranes of PNS and CNS myelin sheaths where it appears to be involved in glia-axon interactions. Recent immunocytochemical observations at the electron microscope level demonstrate its presence in multivesicular bodies of actively myelinating oligodendrocytes, suggesting that it is endocytosed and transported back to the cell body possibly transmitting a signal from the periaxonal region. MAG contains five extracellular immunoglobulin like domains defining it as a member of the immunoglobulin gene superfamily along with other cell-cell adhesion proteins, and in the CNS it occurs in two developmentally regulated forms with different C-terminal tails generated by alternative splicing of the primary mRNA transcript. Although PNS MAG occurs primarily in the shorter form which has only one of four potential phosphorylation sites found in the C-terminus of the longer form, it is heavily phosphorylated in tissue slices and by kinases in purified myelin. The extracellular domains of the two forms of MAG are identical and contain 8 potential sites for N-linked glycosylation. The carbohydrate consists of a mixture of neutral and negatively charged, bi-, tri- and tetraantennary oligosaccharides whose structures are now under detailed investigation utilizing our new Dionex HPLC with a very sensitive amperometric detector. The expression of MAG in cultured oligodendrocytes and Schwann cells is being studied with the ultimate objectives of identifying factors that control its synthesis and probing its function in cell-cell interactions. Cultured oligodendrocytes constitutively express about 0.2 ng of MAG and 1.0 ng of myelin basic protein per ug total protein. Cultured Schwann cells do not normally express MAG but can be induced to express a small amount by elevation of cAMP with forskolin. A spontaneously immortalized Schwann cell line (S-16) generated in our laboratory expresses much more MAG (approx. 1 ng/ug total protein). This continuous cell line in which most of the MAG is on the cell surface should be useful for investigating the function of MAG in cell-cell interactions. Our research on antibodies to MAG and acidic glycolipids in neurological diseases previously reported in this Research Project are covered in a new Project No. Z01NS 02786 beginning with this year.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

N01 NS02710-04LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Neurotransmitter Receptors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.C. Venter, Ph.D., Chief, RBMB, LMCN, NINDS. OTHERS (LMCN, NINDS): C.M. Fraser, Ph.D., Unit Chief; A.R. Kerlavage, Ph.D., Sr. Staff Fellow; J. Gocayne, M.S., Microbiologist; M. Fitzgerald, B.S., Biologist; M.A. Buck, Ph.D., IRTA Fellow; D.A. Robinson, Ph.D., Microbiologist; T. Saverese, Ph.D., Special Volunteer; W. McCombie, Ph.D., Sr. Staff Fellow; S. Arakawa, M.D., Ph.D., Special Volunteer; T. Onai, M.D., Ph.D., Fogarty Fellow; E. Kirkness, Ph.D., Fogarty Fellow; J. Fleming, B.S., Special Volunteer; J. Kusiak, Ph.D., Staff Scientist (NIA); R. Bevan, Ph.D., Special Volunteer.

## COOPERATING UNITS (if any)

Cambridge Univ. (England); Molecular Neurobiology Unit (E.A. Barnerd); DCRT, NIH (R. Feldman); NCI, NIH (H.R. Guy); SUNY at Buffalo (D. Hall, L. Urquhart).

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP, DIR, NINDS

## SECTION

Section of Receptor Biochemistry and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

7.0

## PROFESSIONAL:

6.3

## OTHER:

0.7

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Subjects

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is to characterize the gene and protein structure and evolution of neurotransmitter receptors belonging to two major multi gene families. The gene families are those containing adrenergic, muscarinic, opsin, serotonin and octopamine receptors and the second family containing nicotinic cholinergic, GABA/benzodiazepine and glycine receptors. The specific aims are to clone and sequence the genes for receptors in these two multigene families; to obtain high density receptor expression and to use the expressed proteins to determine the complete receptor structure by x-ray crystallography and computer enhanced molecular modeling; to determine the evolution of the neurotransmitter receptor gene families; and to search for and characterize receptor gene polymorphisms. To date we have cloned and sequenced a significant number of receptor genes from both multigene families. These include beta<sub>2</sub>-adrenergic receptors from human brain cDNA and genomic libraries, rat cardiac cDNA and rat genomic libraries and from shark genomic library; β<sub>1</sub>-adrenergic receptors from a human genomic library; M1-M4 muscarinic cholinergic receptors from human, rat, shark and Drosophila genomic libraries; octopamine receptors from Drosophila; the human α<sub>1</sub>-adrenergic receptor from a human genomic library; human alpha and beta subunits of the GABA/benzodiazepine receptor from human genomic libraries; nicotinic receptor genes from a neuronal cDNA library and from locust cDNA libraries; and a chick genomic clone of the GABA/benzodiazepine receptor. These receptors have been cloned into expression vectors and mammalian cells transfected. The results are permanent cell lines expressing the unique neurotransmitter receptor protein. The expressed receptor lines are providing key new information concerning the mechanism of receptors activated by neurotransmitters and a basis of receptor structural determinants.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS02672-05LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989\*

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotransmitter Receptor Expression and Site Directed Mutagenesis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                        |                    |                                   |
|---------|------------------------|--------------------|-----------------------------------|
| PI:     | C.M. Fraser, Ph.D.     | Unit Chief         |                                   |
| OTHERS: | J.C. Venter, Ph.D.     | Chief, RBMB        | J. Fleming Guest Worker           |
| LMCN,   | M. Buck, Ph.D.         | IRTA               | D. Robinson, Ph.D. Microbiologist |
| NINDS;  | D. Wang, Ph.D.         | Visiting Associate | J. Gocayne, M.S. Microbiologist   |
|         | T. Savarese, Ph.D.     | Special Volunteer  | E. Kirkness, Ph.D. Fogarty Fellow |
|         | J. Giacobino, Ph.D.    | Special Volunteer  |                                   |
|         | S. Arakawa, M.D. Ph.D. | Special Volunteer  |                                   |

## COOPERATING UNITS (if any)

University of Geneva (J.P. Giacobino); SUNY at Buffalo (L.M. Hall, D.A. Urquhart)  
 Cambridge University (England) Molecular Neurobiology Unit (E.A. Barnard, J. Fleming)

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP, DIR

## SECTION

Section of Receptor Biochemistry and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6

## PROFESSIONAL:

5

## OTHER:

1

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The purpose of this project is to achieve stable, continuous expression of neurotransmitter receptor genes in cultured cell lines lacking the receptors. This experimental approach provides a means for 1) definitive identification of cloned neurotransmitter receptor genes using pharmacological and biochemical assays; 2) comparison of the pharmacological and biochemical properties of the same receptor expressed in different cell lines as well as between related receptor proteins; 3) large scale production of receptor proteins; and 4) analysis of the relationship of receptor structure and function using the technique of site-directed mutagenesis without phenotypic selection. To facilitate these goals, we are also constructing new plasmid expression vectors that routinely allow for high density receptor expression.

To date, we have produced several cell lines in murine B-82 cells and Chinese hamster ovary (CHO) cells that are expressing human and rat beta<sub>2</sub>-adrenergic, human beta<sub>1</sub> adrenergic, human alpha<sub>2</sub>-adrenergic, rat M<sub>1</sub>-M<sub>4</sub> muscarinic cholinergic, and Drosophila octopamine receptors at densities in the range of 100 fmol to 10 pmol of receptor/mg membrane protein. These receptors display all of the expected pharmacological and biochemical properties for each respective receptor subtype.

Using site-directed mutagenesis, we have also examined the role of highly conserved aspartate and cysteine residues in beta<sub>2</sub>-adrenergic and muscarinic acetylcholine receptor function. The aspartate and cysteine residues, located in the putative second and third transmembrane domains of the receptors appear to be involved in agonist binding and agonist induced activation of guanine nucleotide regulatory proteins. Extracellular cysteine residues also appear to be involved in agonist and antagonist binding to the receptors, perhaps as important structural elements of the receptor proteins. These data illustrated that the conservation of receptor structure among these related receptors may reflect conservation of receptor mechanisms.

\* (Project transferred to NIAAA with Dr. Fraser on 7-16-89)

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 NS 02754-02 LMCN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Megabase DNA Sequencing

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.C. Venter, Ph.D. Chief, Receptor Biochemistry and Molecular Biology Section, LMCN, NINDS  
 Others: J. Gocayne, M.S., Microbiologist, LMCN, NINDS; D.A. Robinson, Ph.D., Microbiologist, LMCN, NINDS; A.R. Kerlavage, Ph.D., Sr. Staff Fellow, LMCN, NINDS; S. Arakawa, M.D., Ph.D., Special Volunteer, LMCN, NINDS; M. Fitzgerald, B.A., Biologist, LMCN, NINDS; W. McCombie, Ph.D., Sr. Staff Fellow, LMCN, NINDS; T. Onai, Fogarty Fellow, LMCN, NINDS.

## COOPERATING UNITS (if any)

Applied Biosystems Inc. (M. Hunkapiller)

## LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

## SECTION

Section of Receptor Biochemistry and Molecular Biology

## INSTITUTE AND LOCATION

NINDS, NIH, Park Building, Room 405, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.3

## PROFESSIONAL:

2.0

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has as its goal the acquisition and analysis of the sequence of genomic DNA that is associated with receptor multigene families and neurological disorders. To accomplish this, sequencing strategies and technologies are being developed that will allow the sequencing of megabase regions of DNA. Our use of three Applied Biosystems automated DNA sequencers allows the generation of up to 24 kilobases of raw sequence per day. The use of Tag polymerase in the sequencing reactions has greatly enhanced the quality of the sequence data generated as well as the length of the sequence read on each template. We have made a number of technical advances in the past year including 1) improvements in phagemid template preparation procedure, 2) automation of the generation of overlapping deletions with exonuclease III using a Biomek 1000 automated workstation, 3) optimization of the preparation of single stranded DNA template for sequencing, 4) automation of the DNA sequencing reactions using a Biomek 1000 workstation, and 5) the development of computer software to facilitate the transfer of sequence data from the automated sequencers to other computers for assembly and analysis. Work is under way to optimize each of these steps in a way that would allow all of these procedures to be automated. Cosmid clones have been obtained from the human Xq28 region which contains several loci of neurological significance. Clones have also been obtained for the two major classes of neurotransmitter receptor genes, the nicotinic acetylcholine/GABA and muscarinic acetylcholine/adrenergic receptor families. In addition, collaborations have been initiated which will allow clones associated with several important neurological diseases, including neurofibromatosis I and Huntington's Disease, to be obtained and sequenced.







ANNUAL REPORT

October 1, 1988 through September 30, 1989

Biometry and Field Studies Branch  
Division of Intramural Research  
Clinical Neurosciences Program  
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT  
October 1, 1988 through September 30, 1989

Biometry and Field Studies Branch

Division of Intramural Research  
Clinical Neurosciences Program  
National Institute of Neurological Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Chief

The Biometry and Field Studies Branch (BFSB) supports a program in biostatistics to advance the mission of NINDS in the areas of neurologic disorders. The Branch participates in a wide range of intramural and extramural collaborative projects, including large- and small-scale observational studies, clinical trials and laboratory studies. These collaborative studies are conducted both through direct staff research and research and development contracts. In addition to collaborative work, the Branch has an important research component in statistical methodology.

The Branch is composed of an Office of the Chief and three Sections. Dr James Dambrosia is Deputy Chief of the Branch. In that capacity he assists the Branch Chief in policy decisions and assumes part of the administrative burden of running the Branch. The Mathematical Statistics Section, headed by Dr. James Dambrosia is the main statistical consulting unit for other branches and laboratories in the Division of Intramural Research, the Extramural Research Divisions as well as neuroscience units outside of NINDS. This Section is also responsible for research in statistical methodology. The Computer Applications Section is currently primarily occupied with the activities of the Stroke and Traumatic Coma Data Banks. The Branch Chief is Acting Chief of this Section, although Dr. Mary Foulkes is Project Director for both of the Data Banks. With the completion of the enormous data management and data collection activities of the Data Banks, the Section is focusing on analysis of the data from these projects as well as the development of statistical collaborative initiatives for clinical studies in other areas. A change in the name of the Section to the Collaborative Studies Section has been proposed to reflect the current focus of the Section's activities along with the appointment of a permanent Section Chief. The Data Processing Section provides computer programming, systems the analysis, and data management support to the Branch. The Branch Chief is also the Acting Chief of this Section.

Over the past three years, the Branch has aggressively recruited twelve recent Ph.D. statisticians for positions through the Staff Fellow Program. As a result of these efforts, we have been able to attract only one candidate, Dr. Paul Albert of Johns Hopkins University, to accept one of our Staff Fellow positions. Our recruitment efforts to fill vacant positions for entry level biostatisticians have been hampered by increased competition for well-trained Ph.D. level statisticians and a major differential in Staff Fellow salaries as compared to salaries offered by academic institutions and industry.

Several of our current projects are long-term and labor intensive, requiring a large amount of effort for routine activities such as data entry, data editing and day-to-day monitoring of protocol compliance. These activities, although essential, consume an excessive amount of staff time, with the drawback that little time is left for statistical methodological research and expansion of our collaborative efforts into new areas. By the end of FY 1989 most of the data collection, data monitoring and editing aspects of our current large-scale projects will be completed. BFSB collaboration on new large-scale projects with a substantial data management component will be carefully considered; our participation will depend, in large part, on whether routine data management operations of such studies can be contracted out under our supervision. To support ongoing projects and other future collaborative studies, an R&D contract, "Statistical and Collaborative Biomedical Research Data Management Support " (N01-NS-9-2325), was awarded to Information Management Services, Inc. on December 31, 1988. This R&D contract, with an initial funding period of three years, will provide expertise in statistical programming, data entry, data monitoring, and systems analysis, in partial support of collaborative projects and statistical methodological research.

## I. STATISTICAL COLLABORATION AND CONSULTATION

Our current program of collaborative research has developed primarily in response to requests for collaboration from intramural and extramural scientists at NINDS and from researchers outside of NIH. Typically, BFSB assumes responsibility for the statistical design, data management, statistical analysis, and interpretative aspects of the projects, with the subject matter specialists providing the project initiatives, subject matter expertise, and overall leadership. The Branch selects projects on the basis of scientific merit, a high probability of successful completion, and potential for scientific contributions consistent with the goals of the DIR.

In collaboration with the Division of Convulsive, Developmental and Neuromuscular Disorders, (DCDND), BFSB is the statistical coordinating center for the clinical trial of behavioral and cognitive side effects of phenobarbital used for the prevention of febrile seizure recurrence. BFSB has been the comprehensive operations center for this study, which has required extensive monitoring of patient accrual and data quality control and several interim data analyses for the trial's monitoring committee. Patient accrual was completed in December 1985 with 217 children with febrile seizures randomized to treatment and 150 seizure free controls recruited for the study. The two and one-half year follow-up of the last patients was completed in July 1988. The primary results of this clinical trial have been submitted for publication.

A second collaborative effort with the DCDND is a population-based study of the prognostic value of the EEG for subsequent seizure activity in children who experienced a febrile seizure. The cooperating medical center is the Pediatric Clinic in Skopje, Yugoslavia. The recruitment of new cases ended in December 1984, and follow-up (including repeat EEGs and neurologic and physical examinations) is continuing through the end of this calendar year. The study includes 400 children with a normal or non-specific abnormal EEG following a first febrile seizure, as well as about 300 children with a specific abnormal EEG

following a seizure. The major outcomes of the study are recurrent febrile and afebrile seizures and their relationship to the initial EEG, subsequent EEG changes, and the influence of other medical and demographic factors. Univariate statistical analysis of the data for the baseline visit examined a large number of factors predictive of abnormal specific EEG classification. When these factors were considered jointly in a logistic regression model the significant prognostic factors for abnormal specific EEG were: age at initial EEG; number of prior febrile seizures; focal febrile seizures; and motor activity abnormalities.

BFSB is participating with the National Institute of Deafness and Communicative Disorders on a study of factors associated with the acquisition of reading and writing skills by the deaf. Information on audiologic, educational and family background, and on language skills will be examined to determine which, if any, of these variables are associated with reading and writing skills in deaf adolescents. A pilot study was completed and the main data collection phase was completed in early FY 1989. Some research will be required to develop appropriate statistical techniques for measuring the association of background and language factors with reading and writing skills. However, the data collection phase has been extended to allow for additional efforts to obtain certain missing data elements.

The BFSB continues to collaborate with many Branches and Laboratories in the Division of Intramural Research (DIR). The feasibility of a randomized controlled trial of treatment with anti-convulsant medication following a first convulsion in subjects presenting for care to the Beijing Tiantan Hospital is being evaluated. This collaborative study involving BFSB, the Neuroepidemiology Branch, and the Tiantan Hospital in the Peoples Republic of China will address the issue of whether early treatment after a first seizure can reduce the likelihood of developing chronic epilepsy. A similar protocol is being considered for implementation with a consortium of hospitals in Israel. BFSB would collaborate as the statistical coordinating center for these projects.

With the death of Dr. Schoenberg, Chief of the Neuroepidemiology Branch, in June 1987, several BFSB statisticians have taken on the difficult task of collaborating on/or directing the completion of his collaborative projects. A partial list of these projects which involved several visiting scientists include: study of head trauma as a risk factor for Alzheimer's disease; epidemiology of dementia from cerebrovascular disease; geographic distribution of epilepsy and cerebrovascular disease in rural areas of the PRC; examination of the time frame for exposure to environmental risk factors for parkinsonism-dementia complex in Guam; and the incidence of primary intracranial neoplasms in Israel.

A senior statistician, Dr. Anderson, has been providing statistical advice on the planning, design and operations of area surveys for epidemiologic studies of neurologic disorders in Latin America and India. In addition, he is preparing a monograph on the epidemiology of neurologic disorders.

BFSB continues to collaborate with the Medical Neurology Branch on clinical trials of felbamate, a new drug for the treatment of complex partial seizures. The first trial used a randomized, double-blind, three-period-crossover design, allowing unbiased estimation of treatment effect even in the presence of period and carry



over effect. This trial which tested the efficacy of felbamate and carbamazepine given in combination has been completed and a manuscript is in preparation. A second felbamate trial is in the planning stages. In this randomized trial patients will be studied on felbamate alone, and the dosage will exceed that of the initial trial (3000 mg per day).

Other collaborative studies in DIR include: a pilot study of cognitive function in von Recklinghausen's neurofibromatosis (NE); analysis of time to stroke using time dependent covariates in a proportional hazards regression model (NE); a case-control study of the potential association of serologically confirmed infection during pregnancy with morbidity in the child; analysis and comparison of the amplitude of blink responses evoked by mechanical or electrical stimuli for normal controls and spasmodic dysphonic patients (MN); a study of IL-2 and LAC cell therapy for brain tumor in a rat model (SN); an evaluation of stereotactic implantation of dispersed cells into the brain (SN); a case-control study to identify predictors of febrile seizures based on data from six Chinese cities (NE); examination of catecholamine, neuropeptide and amino acid levels in epilepsy patients at baseline and ictal periods (MN); survey of attitude and potential behavior of patients with von Recklinghausen's neurofibromatosis with respect to genetic screening (NE); clustering of occurrences of somatic and affective symptoms in epilepsy patients (MN); nature of parkinsonism-dementia complex on Guam (NE); a review of the epidemiology of movement disorders (NE); prevalence of neurological diseases in the Navajo tribe (MN); hyperarousal in chronic insomnia patients (MN); effect of phenytoin withdrawal on seizure frequency (MN); protective properties of cyclohexyl adenosine against neuronal death following ischemia in the CA1 region of gerbil hippocampus (CNNS); study of epilepsy progression to generalized tonic-clonic seizures (MN); an examination of seizure frequency in patients with intractable complex partial seizures (MN); the effect of dexamethasone suppression tests in medicated patients with poorly controlled partial seizures (MN); clinical evaluation of Ceredase<sup>TM</sup> glucocerebrosidase infusion for treatment of Gaucher's disease (DMN); and a case control study assessing the potential impact of the presence of 11 infections during the second and third trimester of pregnancy on pediatric outcome.

## II. CLINICAL DATA BANKS

BFSB continues its responsibility for the management and operation of the Stroke and Traumatic Coma Data Banks. Each data bank is a collaborative effort between BFSB, which is the statistical coordinating center, and four hospital centers. The data banks were involved in the collection of prospective, observational, clinical and laboratory data at the multiple clinical centers using a common set of data forms. These data banks provided a resource for addressing research questions on the characteristics, clinical course, and outcome of hospitalized stroke and traumatic head injury patients.

Data collection, processing and editing involved the coordinated efforts of BFSB, a contractor for systems analysis and computer programming support (Rush Associates Inc.), and the clinical data bank centers. BFSB designed and implemented a database system and a patient tracking system at NIH (DCRT) to monitor patient accrual and completed follow-up visits. The Rush Associates work scope included design, maintenance, and telecommunications aspects of the

"front-end" micro-computer system as well as transfer of data from the micro-computer to DCRT, and programming support for the building, updating, and editing of the databases at DCRT.

For the duration of both data bank projects resources will be allocated to ensure the continued maintenance, updating and easy access by the principal investigators and BFSB to complete and provide accurate databases. BFSB will continue in its primary scientific leadership role in the collaborative analysis efforts of these projects, providing both statistical collaboration and oversight of the preparation of the scientific reports.

#### Stroke Data Bank

Data collection for the main phase of the Stroke Data Bank began in FY 1983. By the end of new patient accrual in June 1986, 1,805 patients had been entered. All acute care data were entered by the centers, edited and corrected by BFSB and the centers, and a final acute care data file was created in September 1986. Primary analysis of acute care data began at that time. Collection of follow-up data continued until March 1987, and the final edited data base was completed in May 1987. A priority for analysis and publication has been established to focus on the areas of primary interest. A policy for publication and a Publication Committee were established to review and critique all potential publications.

Examples of research studies being addressed include: the identification of patients at risk for evolving ischemic stroke; the investigation of racial and sexual differences in stroke type, site and vascular territory; and the examination of stroke severity to determine whether motor weakness and/or sensory loss can be predicted by the location or size of the CT scan abnormality in infarcts. The risk of dementia among patients initially non-demented has been modeled, showing a strong age-dependence. Trends in institutionalization of post-stroke survivors have been evaluated, with race, sex and marital status predictive of institutionalization. A homunculus profile analysis is in progress which will demonstrate the association, or lack thereof, between lesion location and corresponding motor deficits, using digital mapping of CT scan data on lesion location.

#### Traumatic Coma Data Bank

Data collection for the main phase Traumatic Coma Data Bank (TCDB) began in FY 1984. By the end of September 1987, 1,030 severely head-injured patients were enrolled in this project. Patient follow-up ended in February 1988, and data editing was completed in July 1988.

The first major analyses began after the completion of data collection and editing. The TCDB Steering Committee has identified those primary questions for which the data are the most complete and of the highest quality. Reports being prepared for submission for publication by the end of this year include: outcome following severe head injury; the influence of age on outcome; verbal learning deficits following severe head injury; initial CT scan findings in patients with severe head injury; the impact of intracranial pressure instability and hypotension on outcome; and several methodologic reports on TCDB diagnostic classifications, intracranial pressure monitoring methods, and longitudinal

neurobehavioral assessments following severe head injury. Secondary publications are also in progress, and these include the relationship between intracranial pressure and Glasgow Outcome Score, and post-injury recovery of memory and attention.

### III. METHODOLOGICAL RESEARCH IN STATISTICS

BFSB statisticians continue to develop new statistical methodology and derive innovative modifications of statistical techniques to meet the needs of the Institute for the design of experiments and field studies, analysis of data, and statistical modeling of biological processes and phenomena. Most of the statistical problems addressed arise from collaborative studies with the Intramural and the Extramural Divisions. In general, there are two objectives associated with these various statistical activities of BFSB. The primary objective is the development and improvement of statistical methodology to meet the needs of the Institute. The secondary objective is to make contributions to the development of statistical methodology which may be more generally useful in neurologic and other medical research.

A partial listing of areas in which BFSB staff is developing new statistical applications to neurologic problems includes: modified tests for space-time clustering of rare disease applied to a population in a defined geographic area; methods for adjustment of the effect of concomitant variables in categorical data analysis; sampling strategies for rare neurologic disorders; determination of the order of categories in attribute data; methods of inference on frequency of events in follow-up data; analysis of longitudinal data with missing observations; statistical designs for two-state episodic diseases using follow-up data; and statistical design and analysis of randomized clinical trials in neurology.

Theoretical statistical work has included: the non-null distribution of statistics that measure spatial clustering; the effect of informative and/or prognostic censoring on survival analysis; comparison of common parametric and nonparametric methods for survival analysis in the presence of misspecification; proportional hazard model estimators for transitions in discrete state semi-Markov processes; extension of generalized linear models for random effects; derivation of consistent, efficient estimators for the bivariate Weibull distribution; mixture models for time series count data; and regression models for interval censored time-to-event data.

### IV. BRANCH RECOGNITION AND OTHER PROFESSIONAL ACTIVITIES

Several of our staff have been active on important national and international review committees, and have participated in major meetings or received other peer recognition this fiscal year. Dr. Ellenberg is President of the International Biometric Society. He is also a member of the Parkinsonism Epidemiology Research Committee; the NINDS, Monitoring Committee for the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism Clinical Trial (DATATOP); the NINDS, DIR Clinical Review Board; and the NIH, Ad Hoc Epidemiologist and Statistician Review Panel. Dr. Dambrosia received the NIH Director's Award for "Innovative application of statistics to the design and analysis of research

studies in neurology." Dr. Anderson was appointed to the American Statistical Association's Committee on Committees. Dr. Lee organized and chaired an invited paper session at the Biometric Society Spring meeting. Dr. Foulkes is a member of the Monitoring Committee for the VA Cooperative Study of Carbamazepine versus Valproic Acid for Treatment of Partial Seizures, and gave an invited presentation on the Stroke and Traumatic Coma Data Banks at the University of Vienna. Drs. Ellenberg, Lee, Anderson and Dambrosia were invited chapter contributors for medical monographs.

In summary, BFSB is involved in a strong program of collaborative research. Our collaboration extends throughout the Institute on projects with both Intramural and Extramural scientists, and also involves collaboration with scientists outside of NINDS. The scope of our research activity ranges from small, one-on-one collaboration with intramural scientists, to the conduct of large-scale, multicenter clinical studies. BFSB also makes an important and continuing contribution to statistical methodology applicable to neurological research.



CONTRACT NARRATIVE  
Biometry and Field Studies Branch, CNP, DIR, NINDS  
Fiscal Year 1989

Information Management Services, Inc., Rockville, Maryland  
(NO1-NS-9-2325)

Title: Statistical and Collaborative Biomedical Research  
Data Management Support

Date Contract Initiated: December 30, 1988

Contractor's Project Director: William Lake, Jr.

Current Annual Level FY 89: \$66,840

Objectives: To provide statistical programming and data management support for both collaborative research projects and the development of statistical methodology.

Major Findings: This contract provides statistical programming and data management support for data entry, editing, quality control and report generation for all BFSB collaborative projects. Software for new statistical methods as well as all data management support is developed, tested and implemented by the Contractor on the NIH computer system.

Significance to the NINDS Program and Biomedical Research: The statistical staff of BFSB engages in collaborative biomedical research and conducts statistical research evolving generally from problems encountered in these collaborative studies. The Contract provides timely and efficient systems development, data management (including data entry), data processing and programming for both ongoing and future collaborative studies. Statistical programming, an essential element of biostatistical research, is provided under the direction of the Branch. New statistical methods developed by BFSB are coded, evaluated and then made compatible with existing interactive statistical software packages by the Contractor.

Proposed Course of the Project: The Contract began on December 30, 1988 and continues through December 29, 1991.

Publications: None



CONTRACT NARRATIVE  
Biometry and Field Studies Branch, CNP, DIR, NINDS  
Fiscal Year 1989

1. UNIV. OF TEXAS-GALVESTON (N01-NS-3-2339)
2. UNIV. OF CAL. IN SAN DIEGO (N01-NS-3-2340)
3. MEDICAL COLLEGE OF VIRGINIA (N01-NS-3-2341)
4. UNIV. OF VIRGINIA (N01-NS-3-2342)

Title: Full Phase Traumatic Coma Data Bank

Date Contracts Initiated: 1. April 15, 1983  
2. April 15, 1983  
3. June 1, 1983  
4. July 1, 1983

Contractors' Principal Investigators: 1. Dr. Howard Eisenberg  
2. Dr. Lawrence Marshall  
3. Dr. Harry Young  
4. Dr. John Jane

Current Annual Level FY 1989\*: 1. \$0  
2. \$0  
3. \$0  
4. \$0

\*Final incremental funding for these projects appropriated in FY 1987.

Objectives: The primary objective of this project was to implement the full phase of the Traumatic Coma Data Bank study, which would collect observational acute and long-term longitudinal data on severely head injured patients. The data bank provides a resource for clinical research studies of patients with head injury. This is a collaborative project which involved four clinical centers and BFSB. The clinical centers were responsible for the collection of data and collaboration on the analysis of research questions. The BFSB was the statistical coordinating center, responsible for maintenance of the centralized database management system for transmission, storage and retrieval of data; for monitoring of data acquisition and data quality; and for statistical collaboration or oversight of the statistical analysis for the primary research questions of the data bank.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the initial year of this project, outlined the research objectives, and developed forms and data collection procedures. Data collection began in January 1984. New patient accrual was ended in September 1987, and patient follow-up ended January 1988, with a total of 1,030 patients. During this fiscal year, the focus has been on analysis of data relating to the primary research questions.

(N01-NS-3-2339)  
(N01-NS-3-2340)  
(N01-NS-3-2341)  
(N01-NS-3-2342)

The Publications Committee has been active in the review and prioritization of all potential publications, and in the coordination necessary to provide a logical progression of publications from the TCDB, in a timely fashion. A list of primary publications and their respective writing groups has been established.

Major Findings: Preliminary analyses of the data are ongoing. Reports are being prepared for publication on such topics as: Outcome following severe head injury; the influence of age on outcome; verbal learning deficits following severe head injury; initial CT scan findings in patients with severe head injury; the impact of ICP instability and hypotension on outcome; and a variety of methodologic summaries of TCDB diagnostic classifications, intracranial pressure monitoring methods, and longitudinal neurobehavioral assessments following severe head injury. Additional analyses are also in progress or planned and will take several years to complete.

Significance to the NINDS Program and Biomedical Research: Acute care and longitudinal data on head-injured victims have been collected at four centers, using uniform definitions and procedures. This information will provide a large body of data for clinical research on the factors influencing survival and quality of life following severe head injury. The number of therapies and monitoring devices commonly utilized during the acute phase of managing traumatic coma necessitates a highly organized data handling capacity, and the data bank has served as an efficient mechanism for collecting, storing and retrieving this information as well as follow-up data.

Proposed Course of the Project: Patient follow-up ended in January 1988 and data editing continued through August 1988. Analyses are in progress which will result in a series of Traumatic Coma Data Bank publications. The continued analysis efforts for the TCDB will be subsumed under the Intramural Research Project Traumatic Coma Data Bank (Z01-NS-02516-08). The data collection phase of this project is completed.

Publications:

Marshall SB, Cayard C, Foulkes MA, Hults K, Gautille T, Charlebois DB, Tisdale NA, Turner H. The Traumatic Coma Data Bank: a nursing perspective: Part I. J Neurosci Nurs 1988; 20:253-7.

Marshall SB, Cayard C, Foulkes MA, Hults K, Gautille T, Charlebois DB, Tisdale NA, Turner H. The Traumatic Coma Data Bank: a nursing perspective: Part II. J Neurosci Nurs 1988; 20:290-5.

CONTRACT NARRATIVE  
Biometry and Field Studies Branch, CNP, DIR, NINDS  
Fiscal Year 1989

RUSH & ASSOCIATES, INC., Fairfax, Virginia (N01-NS-6-2305)

Title: Collection and Maintenance of Data for the Stroke Data Bank  
and Traumatic Coma Data Bank Projects

Date Contract Initiated: September 1, 1986

Contractor's Project Director: Robert L. Rush

Current Annual Level FY 1989\*: \$0

\* Final funding for this project appropriated in prior fiscal year.

Objectives: To provide and maintain front-end microprocessor support for the Stroke and Traumatic Coma Data Bank projects (N01-NS-2-2302, 2398-9, N01-NS-5-2384, N01-NS-3-2339-42), with facilities for interactive data entry, updating, editing and transmissions to the NIH DCRT main-frame computer. As well, to provide data maintenance, retrieval and management of the stored data. To maintain and refine software for data monitoring procedures.

Major Findings: This contract provided substantial support for data entry, editing, storage and retrieval for both the Stroke and Traumatic Coma Data Bank projects.

Significance to the NINDS Program and Biomedical Research: The ability to enter and check data on-site via front-end software was an integral part of the Stroke and Traumatic Coma Data Bank projects. This contract also provided the support for data management, storage and retrieval, necessary to fulfill the research objectives of both projects.

Proposed Course of the Project: This contract was completed on April 30, 1989.

Publications: None

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02652-05 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Collaboration and Consultation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James M. Dambrosia, Ph.D. Chief, Mathematical

Statistics Section

BFSB, DIR, NINDS

Others: Paul S. Albert, Ph.D.

Mathematical Statistician

BFSB, DIR, NINDS

Dallas Anderson, Ph.D.

Mathematical Statistician

BFSB, DIR, NINDS

Jonas H. Ellenberg, Ph.D. Chief

BFSB, DIR, NINDS

Sherrie E. Emoto, Ph.D.

Mathematical Statistician

BFSB, DIR, NINDS

Mary A. Foulkes, Ph.D.

Mathematical Statistician

BFSB, DIR, NINDS

Young Jack Lee, Ph.D.

Mathematical Statistician

BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

Department of Statistics and School of Medicine, University of

Rochester, Rochester, New York (R. Raubertas); Bombay Hospital, India (Dr. N.

Bharucha; Dr. Z. Fu, Dr. Z. Zhang, D. S. Li (PRC); Y. Leibowitz (Israel); Dr. J.

de Pedro Cuesta (Sweden).

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

4.1

## PROFESSIONAL:

3.5

## OTHER:

0.6

## CHECK APPROPRIATE BOXES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project encompasses a wide scope of statistical collaboration and consultation with Laboratories and Branches within the Division of Intramural Research (DIR), and with other units outside of NIH. Particular consideration is given to statistical planning and design of experiments, statistical analysis of data, and statistical inference. Our collaboration has involved eight Laboratories/Branches, and the scope of the studies has ranged from the coordination and statistical management of a complex clinical trial to consultation on the appropriateness of the statistical analysis used for small laboratory experiments. Examples of studies with DIR include: randomized clinical trials of felbamate for the treatment of intractable complex partial seizures (MN); comparison of mechanical and electrical evoked blink reflexes in spasmodic dysphonic patients and normal subjects (MN); IL2 treatment of brain tumors in animal models (SN); identification of risk factors for febrile seizures with a population based case-control study of six cities in China (NE); stereotactic implantation of dispersed cells into the rat brain with focus on placement and survival (SN); comparison of tremor in a patient with Parkinson's disease before, at the time of, and after adrenal tissue implant (SN); relationship of behavior and personality to blood and CSF for catecholamine, neuropeptide and amino acid levels in epilepsy patients (MN); clinical course and outcome of patients with Gaucher's disease (DMN); clinical evaluation of Ceredase<sup>TM</sup> glucocerebrosidase in Gaucher's diseases (DMN); prevalence of neurological diseases in the Navajo tribe (MN); use of quasi-likelihood models to demonstrate that seizure frequencies are not random in time (MN); study of epilepsy progression to general tonic-clonic seizures (MN); and the use of hyperarousal scores for diagnosis of chronic insomnia (MN).



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02490-09 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Research in Statistics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |  |                  |
|---------|---------------------------|--|------------------|
| PI:     | James M. Dambrosia, Ph.D. | Chief, Mathematical Statistics Section | BFSB, DIR, NINDS |
| Others: | Paul S. Albert, Ph.D.     | Mathematical Statistician              | BFSB, DIR, NINDS |
|         | Dallas W. Anderson, Ph.D. | Mathematical Statistician              | BFSB, DIR, NINDS |
|         | Jonas H. Ellenberg, Ph.D. | Chief                                  | BFSB, DIR, NINDS |
|         | Sherrie E. Emoto, Ph.D.   | Mathematical Statistician              | BFSB, DIR, NINDS |
|         | Mary A. Foulkes, Ph.D.    | Mathematical Statistician              | BFSB, DIR, NINDS |
|         | Young Jack Lee, Ph.D.     | Mathematical Statistician              | BFSB, DIR, NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.1

## PROFESSIONAL:

2.0

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics. Papers have been submitted or published in FY 1989 on the following statistical subjects: estimation of the joint survival and censoring distribution in the presence of dependent censoring; statistical planning, design and analysis of randomized clinical trials in neurology; case finding and coverage as statistical methodology issues in population-based area studies; general estimation approach for models of longitudinal data; derivation of inferential methods for determining order of categorical data; design of panel studies under alternating poisson process assumptions; and development of statistical methods for the analysis of clinical trial data with non-random missing responses. Other work in progress includes: selection criteria for use of the Kaplan-Meier or parametric MLE for survival analysis; influence of missing data in randomized clinical trials; methods to improve coverage in surveys; analysis of time-to-event data with non-regular censoring; estimation of time-to-event with interval data in the presence of left and right censoring; comparison of parametric and nonparametric survival analysis in the presence of mismodeling; site selection for epidemiological surveys; adjustments for covariates in the analysis of categorical data; and two-state models for analyzing time series count data.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02444-10 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Coordinating Center for the Phenobarbital Clinical Study\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |                           |                   |
|---------|---------------------------|---------------------------|-------------------|
| PI:     | Young Jack Lee, Ph.D.     | Mathematical Statistician | BFSB, DIR, NINDS  |
| Others: | Ta-Chuan Chen, Ph.D.      | Mathematical Statistician | BFSB, DIR, NINDS  |
|         | Jonas H. Ellenberg, Ph.D. | Chief                     | BFSB, DIR, NINDS  |
|         | Deborah G. Hirtz, M.D.    | Pediatric Neurologist     | DNB, DCDND, NINDS |
|         | Dolores Jones             | Computer Assistant        | BFSB, DIR, NINDS  |
|         | Mary F. Livingston        | Clinical Data Assistant   | DNB, DCDND, NINDS |
|         | Karin B. Nelson, M.D.     | Medical Officer           | NEB, DIR, NINDS   |
|         | Jack Panossian            | Programmer                | BFSB, DIR, NINDS  |

## COOPERATING UNITS (if any)

Cerebral Palsy and Other Motor Disorders Section, DNB, DCDND, NINDS;  
University of Washington (Dr. J. Farwell, Dr. S. Sulzbacher)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Biometry and Field Studies Branch is the Statistical Coordinating Center for the ongoing clinical trial of behavioral and cognitive side effects of phenobarbital for prevention of febrile seizure recurrence. The accrual of patients was completed, with 367 patients on study. Follow-up with psychometric testing was completed in July, 1988.

Statistical analyses and a report regarding the primary results of Binet IQ and recurrence of febrile seizures have been completed, and submitted for publication. A large proportion of subjects missed Binet IQ test visits. The missing IQ data were determined not to be missing at random. We developed a statistical analysis method that reduces the magnitude of bias due to the nonrandom missingness. Issues surrounding analysis by "intended treatment" versus analysis by "actual treatment" have been studied. We have shown analysis by actual treatment changes analysis results. We have found that the effective age range of the tests for measuring language development and maturing of play behavior does not extend beyond three years of age. A method for determining adequacy of final sample sizes in the presence of a substantial amount of incomplete data was developed and applied to the assessment of the power of the statistical analysis of recurrence rates of seizures on the different treatment arms. Data management, statistical analysis and manuscript preparation activities will continue into FY 1990. The final master file and analysis files are in place.

\*[This study supports the DNB/DCDND/NINDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINDS, and the contractor for the study is the University of Washington. This project has been subsumed under: Statistical Collaboration and Consultation (Z01-NS-02652-05).]

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02598-07 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stroke Data Bank

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                        |   |                  |
|---------|------------------------|---|------------------|
| PI:     | Mary A. Foulkes, Ph.D. | Mathematical Statistician                 | BFSB, DIR, NINDS |
| Others: | James M. Dambrosia     | Chief, Mathematical<br>Statistics Section | BFSB, DIR, NINDS |
|         | Margaret Meadows       | Statistician Asst.                        | BFSB, DIR, NINDS |
|         | Brenda Dyer            | Programmer                                | BFSB, DIR, NINDS |
|         | Jack Panossian         | Programmer                                | BFSB, DIR, NINDS |
|         | Alan Poliss            | Comp. Sys. Analysis                       | BFSB, DIR, NINDS |

## COOPERATING UNITS (if any)

Departments of Neurology: Boston U. Medical Center, Michael Reese Hospital,  
Neurological Institute - Columbia University, and University of Maryland

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Computer Applications Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

## PROFESSIONAL:

## OTHER:

2.0

1.6

0.4

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreducea type. Do not exceed the space provided.)

The Stroke Data Bank is a prospective observational study which collected data on hospitalized newly diagnosed stroke patients, at four clinical centers. The collaborating clinical centers were responsible for the collection of acute care and longitudinal follow-up information using common definitions and procedures, under contracts N01-NS-2-2302, 2398-9, N01-NS-5-2384. The general objective for the project was to provide a comprehensive body of data for clinical research on the factors influencing survival, morbidity and quality of life following onset of a stroke. The BFSB served as the statistical coordinating center for the project, providing an on-site front-end data entry system with interactive feedback for data editing and the data base management system for transmission, storage and retrieval of data, for monitoring of data acquisition and its quality. The data collection phase was completed in FY'88 including follow-up, with a final cohort of 1805 patients. Now the BFSB is responsible for statistical collaboration with the clinical investigators for the analysis of the primary research questions. The first major analyses began after accrual of patients was completed and the acute care data were entered and edited. An observational study reporting the occurrence within the Stroke Data Bank cohort of dementia both at first exam post-stroke onset and incident dementia over the first year of follow-up summarizes the factors associated with dementia within this cohort. A mathematical model for mortality in ischemic infarction has been developed validating and revising a model originally developed using the pilot Stroke Data Bank. A similar validation model has been developed in intracerebral hemorrhage. An investigation of the predictive value of the CT scan for subarachnoid hemorrhage survival showed that the CT scan result independently predicted outcome after accounting for clinical and medical history.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02516-08 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Traumatic Coma Data Bank

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary A. Foulkes, Ph.D. Mathematical Statistician BFSB, DIR, NINDS

|                          |                    |                  |
|--------------------------|--------------------|------------------|
| Others: Margaret Meadows | Statistician Asst. | BFSB, DIR, NINDS |
| Brenda Dyer              | Programmer         | BFSB, DIR, NINDS |
| Jack Panossian           | Programmer         | BFSB, DIR, NINDS |
| Alan Polis               | Comp. Sys. Analyst | BFSB, DIR, NINDS |

## COOPERATING UNITS (if any)

Depts. Of Neurosurgery: Medical College of Virginia, University of California - San Diego, University of Texas - Galveston, University of Virginia

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Computer Applications Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.8

## PROFESSIONAL:

1.4

## OTHER:

0.4

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The Traumatic Coma Data Bank is a prospective observational study which collected data on severely head injured patients at four clinical centers. The collaborating centers were responsible for the collection of acute care and longitudinal follow-up information using common definitions and procedures, under contracts N01-NS-3-2339-42. The research objectives for the project were formulated by a Steering Committee composed of the principal investigators from the clinical centers, other outside experts, and BFSB staff, with the concurrence of the BFSB Advisory Committee. The research objectives were the basis for determining the specific data to be collected, the format of the data collection forms and the data collection procedures. The general objective for the project was to provide a comprehensive body of data for clinical research on the factors influencing survival, morbidity and quality of life following a severe head injury. The BFSB was the statistical coordinating center for the project, providing an on-site front-end data entry system with interactive feedback for data editing; the data base management system for transmission, storage and retrieval of data, for monitoring of data acquisition and its quality; and for statistical collaboration with the clinical investigators and statistical analysis oversight for the primary research questions of the data banks. Accrual of 1030 patients was completed in September 1987, and patient follow-up was completed in January 1988. Reports are being prepared for publication on such topics as outcome following severe head injury, the influence of age on outcome, verbal learning deficits following severe head injury, initial CT scan findings in patients with severe head injury, the impact of intracranial pressure instability and hypotension on outcome, and several methodologic reports of TCDB diagnostic classifications, intracranial pressure monitoring methods, and longitudinal neurobehavioral assessments following severe head injury.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02590-07 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Studies on the Stroke and Traumatic Coma Data Banks

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary A. Foulkes, Ph.D. Mathematical Statistician BFSB, DIR, NINDS

Other: James M. Dambrosia, Ph.D. Chief, Mathematical  
Statistics Section BFSB, DIR, NINDS  
Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINDS

COOPERATING UNITS (if any) Departments of Neurology: Boston University Medical Center,  
Michael Reese Hospital, Neurological Institute-Columbia University, University  
of Maryland; Departments of Neurosurgery: University of Texas, University of  
California (San Diego), Medical College of Virginia and University of Virginia.

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.30

## PROFESSIONAL:

0.25

## OTHER:

0.05

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project currently includes three studies each of which is a component of the Stroke Data Bank or its precursor, the Pilot Stroke Data Bank. The studies are: (1) Evolving Stroke. Using demographic, history, clinical and laboratory data, this study describes the temporal course of stroke-in-evolution and attempts to identify factors that cause or contribute to evolution. (2) Prognostic factors for 30-day mortality. Multiple logistic regression models, for ischemic stroke and for intracerebral hemorrhage are being used to determine prognostic factors for 30-day mortality. (3) Discrimination between intracerebral hemorrhage and ischemic stroke. This study identifies factors, excluding CT information, available shortly after stroke onset that provide optimal classification into the two diagnostic categories.

Future work on this project will be subsumed under either Intramural Research Project Traumatic Coma Data Bank (Z01-NS-02516-08) or Stroke Data Bank (Z01-NS-02598-07).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02483-09 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Predictive Value of the EEG in Febrile Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sherrie E. Emoto, Ph.D. Mathematical Statistician BFSB, DIR, NINDS

Others: Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINDS  
Deborah G. Hirtz, M.D. Pediatric Neurologist DNB, DCDND, NINDS  
Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS  
Jack Panossian Programmer BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

Cerebral Palsy and Other Motor Disorders Section, DNB, DCDND, NINDS;  
Pediatric Clinic, University of Skopje, Yugoslavia (Nikola Sofijanov)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.50

## PROFESSIONAL:

0.20

## OTHER:

0.30

## CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This population based study will evaluate the significance of the EEG as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcomes reported are febrile seizure recurrence and afebrile seizure occurrence. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study is being carried out in Skopje, Yugoslavia, at the Pediatric Clinic of the University of Skopje.

The study began in FY 1982 and final follow-up visits will be completed in FY 1990. Patient accrual was completed in December, 1984, by which time approximately 400 patients with a febrile seizure, no prior complex or multiple seizures and with a normal or nonspecific abnormal EEG, were registered into the study and began the study protocol and follow-up. An additional 300 patients with a specific abnormal EEG were entered for baseline information and follow-up. Data editing and file creation are continuing. After an on-site review visit, it was determined that additional efforts by the clinical center were needed to collect data from those patients lacking a return visit and those who did not have return visits after 24 months following study entry. This will facilitate study of long-term recurrence of febrile seizures, change in EEG, and the predictive qualities of EEG for febrile seizure occurrence. The extended follow-up effort is now in progress. Statistical analysis of baseline EEG and its association with characteristics of the child and family and the clinical characteristics of the seizure is presently being conducted. The master file, including follow-up data at all visits for all patients, should be completed in FY 1990, at which time further analyses will be undertaken.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02594-07 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Predictive of Reading and Writing Skills in the Congenitally Deaf\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Paul S. Albert, Ph.D. Staff Fellow BFSB, DIR, NINDS

Others: Christy Ludlow, Ph.D. Speech Pathologist DCSD, NIDCD  
Judith Cooper, Ph.D. Speech Pathologist DCSD, NIDCD

## COOPERATING UNITS (if any)

Central Institute for the Deaf, St. Louis, MO (Ann Geers);  
Gallaudet College, Washington, D.C. (Donald Moores)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project consists of the statistical and data editing and file creation aspects of a NIDCD project. Collaboration includes design of data collection and monitoring procedures, and statistical analysis of study data.

The study examined factors that may be associated with development of reading and writing skills in the congenitally deaf. Study subjects comprised three groups of deaf 16- to 17-year-olds, with 65 subjects in each group. Each group included only subjects who received their preschool language training through one of three approaches: aural-oral, total communication, and American Sign Language. Data were collected on the audiologic, familial, and educational background of the subjects, and on their present language skills. Data will be examined for their association with present reading and writing skills of the subjects. Familial and educational data for the main phase have been received and entered onto the NIH computer system. Substantial amounts of missing data dictated the need for new efforts to obtain the information required for the study.

\*[This project is the BFSB/NINDS support of the NIDCD contract study NIH-NINDS-84-19. The project officer is Dr. Judith Cooper, NIDCD.]

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02505-09 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Headache in Pregnant Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ta-Chuan Chen, Ph.D. Mathematical Statistician BFSB, DIR, NINDS

Others: Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

Boston Children's Hospital (Dr. Alan Leviton)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.20

## PROFESSIONAL:

0.15

## OTHER:

0.05

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the relationship between migraine headache and other diseases based on the data collected from the large group of gravidae in the Collaborative Perinatal Project. The report of the investigation of the influence of smoking on associations between migraine and other diseases such as heart and thrombotic diseases and some respiratory and allergic diseases was published in the Archives of Neurology, 1987.

In addition we are examining the possible association of maternal migraine in pregnant women with the health status of their children. Subgroups of women characterized by the absence and presence of migraine and other recurrent headaches prior to or during pregnancy, were identified. Characteristics of these subgroups were examined for a variety of demographic, sociological, medical and obstetric factors, as well as the association of headache with other disorders.

Children of mothers with a history of migraine appear to have higher incidence of some infectious and allergic diseases than children born to mothers in the non-migraine group. Statistical investigation of the latter results has revealed an association of occurrence of bronchial asthma in children born to mothers with migraine, even after partitioning out the potential influence of maternal asthma and allergies. The risk of occurrence of bronchial asthma in children born to mothers with a history of migraine was estimated to be 1.8 times larger than in the children whose mothers did not have migraine headaches. Other factors such as pregnancy complications including placental and/or uterine bleeding disorders, maternal smoking, sex, respiratory infections, and other allergic diseases in children are also being examined for their possible influence on this association. A manuscript is being prepared for publication.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02651-05 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Senile Dementia Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frances M. Baker, M.D., M.P.H. Adjunct Researcher BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.00

## PROFESSIONAL:

0.00

## OTHER:

0.00

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In conjunction with the Baltimore site of the Epidemiologic Catchment Area Survey of NIMH, NINDS funded a detailed dementia workup on those persons in the elderly sample identified with dementing illness, by comprehensive psychiatric examination. The detailed dementia workup included a neurologic examination and comprehensive laboratory studies which included thyroid function tests, electrolytes, BUN and glucose, B12 and folate levels, calcium and phosphorous levels, syphilis serology, urinalysis, chest X-ray, EKG, EEG, and CT scan. The foci of this investigation were (1) to explore the limitations of the Mini-Mental-State-Examination (MMSE) as a screening instrument, (2) to explore which components of the comprehensive dementia workup provide the highest specificity when used as a dementia screening test, (3) to develop a screening model for dementia with a sensitivity and specificity improved beyond that of the MMSE, and (4) to examine the usefulness of this model in other elderly populations. In addition, for the confirmed cases of dementia, additional information was gathered to assess the social and economic impact upon the caregivers.

A review of the data indicated that only 36 confirmed cases of dementia were identified and available for further analysis. The sample size was not adequate to address the four areas of interest and to develop a model for dementia screening beyond the MMSE. After review of the information on social and economic impact, it was determined that the study of the burden on caregivers was also not feasible. Since the findings were inconclusive, no publications are planned and this project is completed.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02654-04 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Incidence of Mental Retardation (MR) in Olmstead County

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frances M. Baker, M.D., M.P.H. Adjunt Researcher

BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

Neuroepidemiology Branch, DIR, NINDS; Mayo Clinic, Department of Health Science Research (Leonard Kurland, M.D.)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

0.0

## PROFESSIONAL:

0.0

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In conjunction with Dr. Leonard Kurland of the Mayo Clinic in Rochester, Minnesota, the birth records and school records of all Olmstead County residents born in 1960 and 1961 will be reviewed. All persons with mental retardation will be identified from these sources. Mental retardation (MR) is defined for the purpose of this study, as a fixed cognitive deficit which occurred before age 12 with some impairment of social adaptation. In addition to the specification of the incidence and identification of risk factors for MR in this birth cohort, service utilization by the MR cases will be assessed. Specific services which will be assessed will include medical (psychiatric, neurologic, gynecologic, and surgical), social (group homes, sheltered workshops, and rehabilitative programs), and educational (special program or projects and institutional placements).

The long-term follow-up of the "9-3" mentally retarded child will be described and mechanism for prediction of functional level in adulthood will be investigated.

This study was discontinued temporarily due to funding constraints. It has been resumed as a collaborative project with the Mayo Clinic and the University of Texas Health Science Center (Dr. Baker). This project is completed.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02719-03 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Symptoms in Alzheimer's Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frances M. Baker, M.D., M.P.H. Adjunct Researcher BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

Mayo Clinic, Department Health Science Research (Leonard T. Kurland, M.D., Emre Kokman, M.D.)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.0

## PROFESSIONAL:

0.0

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

With prior cooperation of Drs. Emre Kokman, Vijay Chandra, and Bruce Schoenberg, all incident cases of Alzheimer's Disease (AD) identified between 1965 and 1974 were made available to the Principal Investigator for three studies on this sample of 268 cases of clinically diagnosed AD. All medical records as well as state hospital records and nursing home records will be reviewed in order to identify psychiatric symptoms and psychiatric disorders which occurred in these patients throughout their lifespan. These symptoms and disorders will be divided into those occurring pre- and post- the onset of AD. A second study which will require review of all hospitalizations to identify all episodes of delirium (nocturnal disorientation) which occurred, will classify these episodes as pre- or post- the onset of AD. A third study will require the identification of all patients with clinically diagnosed AD and a major depressive episode (MDE). These will serve as the cases in a case-control study assessing MDE as a precursor or predisposing factor for AD. The two sets of controls will be identified from 1) age, sex, and race-matched controls with AD and no prior history of a MDE and 2) "normal controls" defined as matched for age, sex, and race, who do not have AD.

A manuscript "Psychiatric symptoms in cases of clinically diagnosed Alzheimer's disease" has been submitted for publication and will be reported under Intramural Project Statistical Collaboration and Consultation (Z01 NS 02652-05). This project is completed.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02506-09 BFSB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibody Titers in Macacas on Cayo Santiago

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James M. Dambrosia, Ph.D. Deputy Chief BFSB, DIR, NINDS

Other: William T. London, D.V.M. Chief, Experimental IDB, DIR, NINDS  
Pathology Section

## COOPERATING UNITS (if any)

Animal Health and Care Section, DIR, NINDS; Caribbean Primate Research Center,  
University of Puerto Rico (Matthew J. Kessler, Project Director)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

## PROFESSIONAL

## OTHER:

0.05

0.05

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project tested for the presence of several viral antibodies in adult and juvenile Macacas on Cayo Santiago, Puerto Rico. This has been a closed colony of Rhesus monkeys since 1938. A serological screen carried out in the early 1950's indicated the presence of antibody to SV 40 (46%), herpes B (27%) and measles virus (80%) of the animals in the colony. The objective was to determine, after 40 years as a closed colony, if herd immunity to the three previously studied antigens has been lost, and thus provide an animal population useful for the testing of related strains of viruses. With the tremendous interest and need for animal models of AIDS and other human diseases, the following viral antigens were added to the testing protocol: human T-lymphotropic virus I (HTLV-I), human immunodeficiency virus (HIV), rhesus CMV, simian retrovirus D (SRV-I) and simian T-lymphotropic virus type I (STLV-III). In addition, the scope of testing was increased to include animals in the NINDS breeding colony in Puerto Rico.

The percent of animals positive for SV40, rubeola, rhesus CMV, HTLVI and herpes-B were: 0.5%, 10.3%, 97.1%, 72.5% and 81.4%, respectively. All animals were serologically negative for SRV-I, STLV-III and HTLV-III (HIV) and are appropriate for research studies in AIDS. A manuscript reporting the results has been accepted for publication. This project has been completed.

Publications:

N01-NS-3-2339  
N01-NS-3-2340  
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Z01 NS 02652-05 BFSB

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Z01 NS 02490-09 BFSB

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989  
Developmental and Metabolic Neurology Branch  
Clinical Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989  
Developmental and Metabolic Neurology Branch, DIR  
National Institute of Neurological Disorders and Stroke

Roscoe O. Brady, M.D., Chief

Principal activities of the Branch concern the following areas of investigation: 1. Basic studies of sphingolipid and mucopolysaccharide synthesis, catabolism, and enzymatic abnormalities in heritable human metabolic disorders. 2. Investigations of the molecular basis of metabolic storage disorders. 3. Pathogenic and therapeutic investigations in animal models of disorders of metabolism. 4. Development of transgenic animal models of human disorders. 5. Clinical investigations of lysosomal storage disorders and neurogenetic diseases. 6. Development of therapy for patients with heritable diseases. 7. Development of gene replacement technology.

### I. BASIC INVESTIGATIONS IN HEREDITARY METABOLIC DISORDERS

#### A. Gaucher's disease

Fundamental studies are underway to examine the effects of lipid activators and small molecular weight protein "cohydrolases" on sphingolipid degrading enzymes. It is anticipated that appropriate combinations of these substances will improve the efficiency of exogenous enzymes administered to patients with lipid storage disorders. Maximally effective mixtures of these agents have been identified and their application to the treatment of Gaucher's disease is under consideration.

#### B. Types C and D Niemann-Pick Disease

We have been able to reverse the intracellular accumulation of LDL-derived unesterified cholesterol in cultured skin fibroblasts obtained from patients with Type C Niemann-Pick disease by adding dimethylsulfoxide (DMSO) to the tissue culture fluid. The mechanism by which DMSO exerts this effect is under investigation. Additional studies are directed at efforts to determine the underlying molecular defect in Type C Niemann-Pick disease.

#### C. Fabry's disease

Studies have been carried out on the targeting of exogenous ceramidetrihexosidase to cells in which ceramidetrihexoside accumulates in patients with this disorder. This strategy has been successfully employed in enzyme replacement for Gaucher's disease, and its applicability to enzyme replacement therapy for patients with Fabry's disease will be investigated.



## II. INVESTIGATIONS OF THE MOLECULAR BASIS OF METABOLIC STORAGE DISORDERS

### A. Type C Niemann-Pick disease

Considerable progress has been made in our attempts to unravel the molecular basis of this disorder. Much current effort is focused on the possibility that a mutation has occurred in an intracellular cholesterol transport protein.

## III. PATHOGENIC AND THERAPEUTIC INVESTIGATIONS IN ANIMAL MODELS

### A. Murine Analog of Type C Niemann-Pick Disease

The treatment of patients with Type C Niemann-Pick disease currently devolves from the concept that cholesterol is the principal offending metabolite in the organs and tissues of patients with this metabolic disorder. Investigations with a spontaneously occurring murine analog of the human disease revealed that modifying the quantity of dietary cholesterol can affect the life span of affected mice. Restricting cholesterol leads to increased longevity whereas supplemental dietary cholesterol hastens morbidity and mortality. These studies provide support for our attempts to alter cholesterol burden in patients with Type C Niemann-Pick disease.

## IV. DEVELOPMENT OF TRANSGENIC ANIMAL MODELS OF HUMAN DISORDERS

### A. Gaucher's Disease

Progress has been made in developing a transgenic murine model of Gaucher's disease. Genomic clones of the mouse glucocerebrosidase have been produced. The nucleotide and deduced amino acid sequences of mouse glucocerebrosidase have been found to be 82 percent and 86 percent identical to the respective human sequences. The glucocerebrosidase gene is on chromosome 3 in the mouse and on chromosome 1 in humans. Retroviral constructs are being made for the production of a transgenic mouse analog of human Gaucher's disease.

### B. Sickle Cell Anemia

Transgenic mice will be generated that contain the abnormal Antilles  $\beta$ -sickle globin gene. The human  $\alpha$ -globin gene is also inserted in order to generate mice that produce high levels of  $\beta$ -sickle Antilles and human  $\alpha$ -globins. After generating these mice, the transgenic mice will be mated with  $\alpha$ -thalassemic mice to eliminate the mouse globins that disturb formation of sickle polymers. Proper utilization of these mice will greatly accelerate investigations of therapeutic agents designed to correct sickling.

### C. Transgenic Mice Containing a Defective IL-3 Gene

A principal objective of this investigation is to determine the consequences of altering the functional state of genes that control cell growth and maturation. This will be attempted through various studies, including an effort to generate transgenic mice with a defective IL-3 gene. If such animals can be produced, attempts will be made to correct the induced growth and maturation problems by homologous recombination with a normally functioning IL-3 gene.

## V. CLINICAL INVESTIGATIONS OF METABOLIC DISORDERS AND NEUROGENETIC DISEASES

### A. Familial Diurnally Variant Dystonia

Genetic linkage studies and an examination of the mode of inheritance associated with low cerebrospinal fluid bipterin levels are underway in the Clinical Investigations and Therapeutics Section at this time. A beneficial effect of supplemental bipterin has been observed in one of these patients.

### B. von Hippel-Lindau Disease

Extensive investigations have been carried out on the genetic linkage and pathogenesis of this heritable condition. Improved diagnostic and prognostic tests have been developed for counseling patients and families in which this disease occurs.

### C. Fabry's Disease

Corrective therapy has been devised for the autonomic nervous system dysfunction that occurs in patients with this disorder. In particular, treatment regimens are being devised that relieve the painful acroparesthesias without exacerbating signs and symptoms of autonomic dysfunction. Additional studies are directed toward correcting gastric hypomotility in Fabry patients.

### D. Lennox-Gastaut Syndrome

Potential metabolic alterations in patients with this syndrome are under investigation. Two patients with this disorder that exhibit non-ketotic hyperglycinemia have been identified. Such studies may lead to the development of specific remedial therapy in patients with underlying metabolic defects with this clinical presentation.

### E. Type C Niemann-Pick Disease

The incidence of seizures and the occurrence of cataplexy has been determined in patients with this disorder. The effect of modifying dietary cholesterol and responses to drugs that alter cholesterol production and its intracellular disposition are under investigation in Type C Niemann-Pick patients.

## VI. DEVELOPMENT OF THERAPY FOR HERITABLE DISORDERS

### A. Gaucher's Disease

1. We have nearly completed a pharmacodynamic investigation of the administration of varying quantities of glucocerebrosidase targeted to macrophage storage cells to patients with Gaucher's disease. We have observed both reduction of hepatic glucocerebrosidase and salutary ultrastructural changes following infusion of the enzyme. The threshold at which these changes can be detected is being evaluated.

2. A major trial has been initiated to determine the clinical efficacy of prospective infusions of mannose-terminated human placental glucocerebrosidase. Parameters being examined include hematologic status, organ size and function, as well as skeletal changes.

### B. Type C Niemann-Pick Disease

A Phase-I clinical protocol has been initiated to examine the therapeutic potential of oral administration of dimethylsulfoxide (DMSO) to patients with Type C Niemann-Pick disease. In addition, the effects of other agents known to modify organ and tissue cholesterol levels will be examined alone and in combination with DMSO in this study.

## VII. DEVELOPMENT OF GENE REPLACEMENT TECHNOLOGY

### A. Gaucher's disease

High-titer recombinant retroviruses have been constructed containing the human glucocerebrosidase gene. The gene has been efficiently transferred into progenitor cells and repopulating stem cells of mouse bone marrow. It is expressed at RNA and protein levels in progeny of CFU-S multipotential progenitor cells following gene transfer. The gene has also been transferred to human hematopoietic cell lines and progenitor cells. Glucocerebrosidase activity has been increased to normal levels following transfer into hematopoietic progenitor cells from Gaucher patients. These results provide a sound experimental basis for continued exploration of gene replacement for the treatment of patients with Gaucher's disease.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Division of Intramural Research, NINDS  
October 1, 1988 through July 31, 1989

Contractor: WEIZMANN INSTITUTE OF SCIENCE (N01-NS-6-2399)

Title: Production of Radiolabeled Glycolipids and Other Sphingolipid Derivatives.

Contractor's Project Director: Ora Goldberg, Ph.D.

Current Annual Level of Support: \$73,425

Objectives: To prepare glucocerebroside, sphingomyelin, and ceramidetrihexoside labeled with  $^{14}\text{C}$  in critical portions of the molecule for diagnostic tests for Gaucher's disease, Niemann-Pick disease, and Fabry's disease.

Major Findings: The Weizmann Institute of Science has extensive and recognized expertise in the chemical synthesis of sphingolipids. Procedures have been developed to incorporate radioactive carbon- $^{14}$  into specific portions of sphingolipid molecules. These compounds are used to diagnose patients with the sphingolipid storage disorders listed above, to identify heterozygous carriers of these conditions, to diagnose these disorders prenatally, and to monitor enzyme isolation procedures for glucocerebrosidase, sphingomyelinase, and ceramidetrihexosidase.

Significance to Biomedical Research and to the Program of the Institute: The ability to diagnose patients, identify heterozygotes, and monitor pregnancies at risk for sphingolipid storage disorders represents major contributions to the control of the incidence of these diseases. These procedures are in wide use at the present time.

Proposed Course of the Contract: The contract has been terminated. It will be recompeted in order to have the requisite radiocarbon labeled sphingolipids available for the Branch's research.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Division of Intramural Research, NINDS  
October 1, 1988 through September 30, 1989

Contractor: GENZYME CORPORATION, BOSTON, MA. (NO1-NS-6-2304)

Title: Preparation of Glucocerebrosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: \$390,000

Objectives: To isolate human placental glucocerebrosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Gaucher's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental glucocerebrosidase targeted to cells in which glucocerebrosidase accumulated in patients with Gaucher's disease. The intravenous infusion of this enzyme causes a decrease in the quantity of glucocerebrosidase stored in the liver and in association with erythrocytes in the blood. Long-term administration of the enzyme has caused significant hematologic improvement in a patient with Gaucher's disease.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. The results indicated in the preceding paragraph will be extended with the hope of providing widely useful treatment for human genetic diseases.

Proposed Course of the Contract: We have investigated procedures to stabilize and to target the enzyme to the specific cells in which toxic quantities of lipid accumulate. We are also developing additional methods to increase the efficiency of this enzyme in vivo.



## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Division of Intramural Research, NINDS  
October 1, 1988 through September 30, 1989

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-6-2301)

Title: Preparation of Ceramidetrihexosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: \$110,000

Objectives: To isolate human placental ceramidetrihexosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Fabry's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental ceramidetrihexosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Fabry's disease. Its carbohydrate portion has been analyzed. We are developing methods to target the enzyme to cells and tissues where ceramidetrihexoside is stored.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy for human diseases. If salutary clinical results can be obtained, an extraordinary milestone will have been accomplished regarding this type of a human genetic disease.

Proposed Course of the Contract: We have initiated experiments to increase the delivery of this enzyme to specific cells in which ceramidetrihexoside accumulates. When this investigation is completed, we shall reinstate enzyme replacement trials in patients with Fabry's disease. We shall examine the effectiveness of the enzyme with regard to clearance of accumulated ceramidetrihexoside in the liver, kidney and in the blood, and monitor clinical responses in this therapeutic trial.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Division of Intramural Research, NINDS  
July 16, 1989 through September 30, 1989

Contractor: GENZYME CORPORATION, BOSTON, MA. (NO1-NS-9-2360)

Title: Preparation of Sphingomyelinase from Human Placental Tissue

Contractor's Project Director: F. Scott Furbish

Current Annual Level of Support: \$100,000

Objectives: To isolate human placental sphingomyelinase in sufficient purity and quantity for use in enzyme replacement trials in patients with Niemann-Pick disease.

Major Findings: A procedure is being developed for the large-scale isolation of human placental sphingomyelinase. Its specific catalytic activity will be such that its final purification stages can be performed at NIH. The highly enriched enzyme will be administered to patients with Type B Niemann-Pick disease and its effect on liver and blood sphingomyelin levels will be determined.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy for human diseases. If salutary biochemical results can be obtained, a clinical trial will be conducted to determine the efficacy of enzyme replacement in patients with Type B Niemann-Pick disease.

Proposed Course of the Contract: We have carried out experiments to isolate human placental sphingomyelinase in a high degree of purity. Large quantities of a partially purified preparation are required in order to obtain sufficient enzyme for an investigation of its effect on stored sphingomyelin in Niemann-Pick patients. When a sufficient quantity of pure sphingomyelinase is available, we shall determine if it causes a reduction of accumulated lipid in the liver and blood of patients with this disorder.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Division of Intramural Research, NINDS  
October 1, 1988 through September 30, 1989

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-8-2317)

Title: Preparation of  $\beta$ -galactosidase from Human Placental Tissue

Contractor's Project Director: F. Scott Furbish

Current Annual Level of Support: \$65,000

Objectives: To isolate human placental  $\beta$ -galactosidase in sufficient purity and quantity to determine its effect on ganglioside  $G_{M1}$  in the brain of cats with generalized ( $G_{M1}$ ) gangliosidosis.

Major Findings: A procedure is being developed for the large-scale isolation of human placental  $\beta$ -galactosidase. The purified enzyme will be injected intra-arterially into cats with the feline analog of human generalized ( $G_{M1}$ ) gangliosidosis after temporarily opening the blood-brain barrier.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy for human diseases. If the injected  $\beta$ -galactosidase causes a significant reduction of ganglioside  $G_{M1}$  in the central nervous system of the affected cats, it will provide incentive to explore enzyme replacement in humans with metabolic storage disorders that involve the central nervous system.

Proposed Course of the Contract: We shall infuse purified placental  $\beta$ -galactosidase after opening the blood-brain barrier in cats with generalized ( $G_{M1}$ ) gangliosidosis. Brain biopsy samples taken before and after administration of the enzyme will be analyzed for their ganglioside  $G_{M1}$  content.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00815-29 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Complex Lipids of Nervous Tissue

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                |              |     |       |
|---------|----------------|--------------|-----|-------|
| PI:     | R. O. Brady    | Chief        | DMN | NINDS |
| OTHERS: | P. G. Pentchev | Biochemist   | DMN | NINDS |
|         | R. R. O'Neill  | Staff Fellow | DMN | NINDS |
|         | J. M. Quirk    | Chemist      | DMN | NINDS |

## COOPERATING UNITS (if any)

Laboratory of Cellular and Developmental Biology, NIDDKD, Laboratory of Biochemistry, Faculty of Medicine, Lyon-Sud, France

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Enzymology and Genetics, Molecular and Cellular Pathophysiology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6

## PROFESSIONAL:

5.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The metabolic defect in patients with Types C and D Niemann-Pick disease has been shown to be due to abnormal intracellular cholesterol homeostasis. The molecular lesion in these disorders results in: (1) failure to down-regulate LDL receptors on cell membranes; (2) lack of down-regulation of HMGCoA reductase, a key enzyme in cholesterol biosynthesis; and (3) inability to up-regulate acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the esterification of intracellular cholesterol. Tests have been developed and introduced into medical practice for the diagnosis of Types C and D Niemann-Pick disease and the identification of heterozygotes, and the prenatal diagnosis of these conditions. Current emphasis is on the development of effective therapy for patients with this disorder.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS-02162-15 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Compounds Analogous To Glycolipids

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:

S. P. Miller

Sen. Staff Fellow

DMN NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Neurochemical Methology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Summary: A synthetic project was initiated this year involving a new type of glycolipid analog. The object of this work is to prepare artificial substrates of sphingolipid hydrolases that can detect enzyme activity within intact cells. Chromogenic substrates synthesized earlier in this section have proven highly useful for enzyme measurements *in vitro*. However, the requirements of *in vivo* measurement of enzyme activity necessitate the preparation of fluorogenic glycolipids. The substrates required for such an approach should be nonfluorescent prior to enzymatic cleavage of the glycoside bond. The released product should then be fluorescent at lysosomal pH. A group of strongly fluorescent phenols, obtained by 0-4 monoalkylation of 2,3-dicyanohydroquinone, were synthesized for this project. These were elaborated into glycosides which were confirmed to be nonfluorescent. The glucose derivative was shown to be a highly active substrate for human glucocerebrosidase. It was hydrolysed *in vivo* 3-5 fold faster by normal fibroblasts in comparison to fibroblasts cultured from a Gaucher Type I patient. The primary use for these probes will be in conjunction with glucocerebrosidase gene-transfer experiments. In this setting, *in vivo* fluorogenic substrates have the potential to greatly increase the sensitivity for detection of glucocerebrosidase expression in transformed cells. In conjunction with fluorescence activated cell sorting, fluorogenic probes should also expand the possibilities for study of the developmental biology of these recombinant cells. Extension of this project to include galactosidase substrates is in progress.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01457-23 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Chemical Synthesis of Radioactive Sphingolipids

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. E. Gal  
OTHER: S. P. MillerSection Chief  
Sen. Staff FellowDMN  
DMNNINDS  
NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Neurochemical Methodology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

. Project terminated, principal investigator retired, 4/30/89.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02163-15 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Analytical Methods for the Use of Research of Sphingolipidoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. P. Miller

Sen. Staff Fellow

DMN

NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Neurochemical Methodology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. A novel clinical presentation involving accumulation of ceramide lactoside in various organs with particularly severe kidney damage was analyzed and the salient findings reported.

A second previously undisclosed clinical syndrome was documented that is characterized by xanthomata and accumulation of bis(monoacyl)glycerol phosphate in the liver. The results of this investigation have also been published.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02435-10 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Mucopolysaccharidoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Constantopoulos

Research Chemist

DMN

NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Enzymology and Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.9

## PROFESSIONAL:

0.9

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project Terminated, principal investigator retired, 4/30/89.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02453-09 DMN

PERIOD COVERED

October 1, 1938 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gaucher's Disease: Biochemical and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |             |                   |     |       |
|---------|-------------|-------------------|-----|-------|
| PI:     | R. O. Brady | Chief             | DMN | NINDS |
| OTHERS: | N. Barton   | Section Chief     | DMN | NINDS |
|         | G. Murray   | Special Volunteer | DMN | NINDS |
|         | G. Zirzow   | Biologist         | DMN | NINDS |
|         | C. Argoff   | Med. Staff Fellow | DMN | NINDS |
|         | J. Fink     | Sen. Staff Fellow | DMN | NINDS |
|         | S. Reisz    | Special Volunteer | DMN | NINDS |

COOPERATING UNITS (if any)

Massachusetts Gen. Hospital, Dept. of Orthopedic Surgery, Boston, MA: (H. Mankin, D. Rosenthal, S. Doppelt); Children's Hospital, Washington, D. C. (P. Guzzetta)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Clinical Investigations & Therapeutics Section, and Enzymology & Genetics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.5

PROFESSIONAL:

3.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Therapy for Gaucher's disease depends upon broad clinical and basic scientific knowledge of the disorder. Patients have been extensively studied and complications identified. Research on glucocerebrosidase addresses the biochemistry, cell biology, and molecular genetics of the enzyme. A pharmacodynamic assessment of mannose-terminated human placental glucocerebrosidase has been completed. The results obtained in this study provide the basis for a clinical efficacy trial in Gaucher patients with this enzyme that is targeted to lipid storing cells. This investigation is currently in progress.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02619-06 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oxidative Metabolism in Patients  
with Inherited Neurological Diseases and in Mollicutes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Constantopoulos

Research Chemist

DMN

NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental &amp; Metabolic Neurology

## SECTION

Enzymology and Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.8

## PROFESSIONAL:

0.4

## OTHER:

0.4

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated, principal investigator retired, 4/30/89.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02648-05 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Human Glioma and Rat Prostate Adenocarcinoma Cells In Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                            |                  |     |       |
|----------------------------|------------------|-----|-------|
| PI: George Constantopoulos | Research Chemist | DMN | NINDS |
| OTHERS: R. Brady           | Chief            | DMN | NINDS |
| C. Kaneski                 | Biologist        | DMN | NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental &amp; Metabolic Neurology

## SECTION

Enzymology and Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.8

## PROFESSIONAL:

0.4

## OTHER:

0.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated, principal investigator retired.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02657-05 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Molecular and Genetic Studies of Niemann-Pick Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Barton  
Other: K. OliverSection Chief  
BiologistDMN  
DMNNINDS  
NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Clinical Investigations and Therapeutics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.9

## PROFESSIONAL:

0.3

## OTHER:

0.6

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Niemann-Pick disease is a progressively debilitating, neurogenetic disorder which is characterized biochemically by the accumulation of sphingomyelin in several tissues and organs in conjunction with deficiency of the lysosomal hydrolase, sphingomyelinase. Detailed description of various phenotypes in terms of cellular pathochemistry and molecular genetics has not been accomplished to date. A major obstacle in this area has been the absence of reproducible techniques for the isolation of homogeneous preparations of sphingomyelinase. Employing novel detergent and chromatography systems, we have purified sphingomyelinase to homogeneity. The purified enzyme migrates with an apparent molecular weight of 67,000 daltons in SDS-polyacrylamide gels under both reducing and nonreducing conditions. Kinetic analyses and determinations of the primary protein structure and carbohydrate composition are in progress. Polyclonal antibodies have been raised to the purified enzyme that will assist in cloning the gene for sphingomyelinase. Characterization of the phenotypes of Niemann-Pick disease in terms of protein polymorphisms and specific mutations at the DNA level will be undertaken.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02664-05 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Clinical Studies of Neurogenetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |                   |     |       |
|---------|-----------------|-------------------|-----|-------|
| PI:     | N. Barton       | Section Chief     | DMN | NINDS |
| Others: | R. Brady        | Chief             | DMN | NINDS |
|         | J. Fink         | Med. Staff Fellow | DMN | NINDS |
|         | M. Filling-Katz | Med. Staff Fellow | DMN | NINDS |
|         | J. Sokol        | Special Expert    | DMN | NINDS |
|         | F. Merrick      | Med. Staff Fellow | DMN | NINDS |

## COOPERATING UNITS (if any)

Armed Forces Institute of Pathology, (K. Ishak);  
Massachusetts General Hospital, (H. Mankin and S. Doppelt)

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Clinical Investigations &amp; Therapeutics Section/Enzymology and Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

7.0

## PROFESSIONAL:

6.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

A number of novel neurogenetic diseases have been identified by studies carried out under this project. Genetic linkage studies are underway in familial diurnally variant dystonia associated with low CSF biopterin levels and a beneficial effect of supplemental biopterin has been documented in one of these patients. Extensive studies of the pathogenesis of von Hippel-Lindau disease have been performed. Corrective therapy has been developed for autonomic nervous system dysfunction associated with Fabry's disease. Potential metabolic alterations in the Lennox-Gastaut syndrome have been examined. The incidence of seizures and cataplexy has been determined in patients with Type C Niemann-Pick disease. The effect of modifying dietary cholesterol and the responses of patients to drugs that alter cholesterol synthesis are under investigation in this disorder.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

ZU1 NS 02730-03 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Globin Genes into Hematopoietic Progenitor and Stem Cells of Mice Retroviral Mediated Transfer of

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |             |               |     |       |
|---------|-------------|---------------|-----|-------|
| PI:     | S. Karlsson | Section Chief | DMN | NINDS |
| OTHERS: | D. Bodine   | Staff Fellow  | CHB | NHLBI |
|         | L. Perry    | Biologist     | DMN | NINDS |
|         | A. Nienhuis | Chief         | CHB | NHLBI |

## COOPERATING UNITS (if any)

Dept. of Hematology, University of Washington (T. Papayannopoulou)

## LAB/BRANCH

Developmental &amp; Metabolic Neurology

## SECTION

Molecular &amp; Medical Genetics Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS

2.3

## PROFESSIONAL:

1.5

## OTHER:

0.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Retroviral vectors can be used to transfer gene effectively into cell lines and primary cells. Less is known, however, whether these vectors can be used to transfer genomic genes into tissue culture cells and animals to yield tissue specific expression of the transferred gene. We have now shown, that retroviral vectors carrying the human beta-globin gene can lead to regulated expression of human globin RNA and protein in murine erythroleukemia cells. In addition, we have recently designed a new high titer ecotropic retroviral vector that has been used to transfer the human beta globin gene into CFU-S murine spleen colonies. The human beta globin gene is expressed in a vast majority of the CFU-S colonies indicating preferential integration at transcriptionally active sites. The expression level of the transferred human beta globin gene was found to be 1-5% of the mouse beta globin genes.

Infected bone marrow was transplanted into genetically anemic W/W<sup>y</sup> mice resulting in production of human beta globin chains in circulating red cells of long term reconstituted mice. The human beta globin gene is detected in all hematopoietic lineages of these mice and it is expressed in a tissue-specific manner. Bone marrow from a primary recipient that had contained the beta globin gene for a whole year was transplanted into a secondary recipient. The secondary recipient contains and expresses the human beta globin gene in erythroid cells more than three months after transplantation proving that the initial gene transfer was targeted to repopulating bone marrow stem cells.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02731-03 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Inherited Enzyme Deficiencies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |             |                   |     |       |
|---------|-------------|-------------------|-----|-------|
| PI:     | S. Karlsson | Section Chief     | DMN | NINDS |
| OTHERS: | J. Fink     | Med. Staff Fellow | DMN | NINDS |
|         | L. Perry    | Biologist         | DMN | NINDS |
|         | P. Correll  | Special Volunteer | DMN | NINDS |
|         | R. Brady    | Chief             | DMN | NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Molecular and Medical Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6.0

## PROFESSIONAL:

3.5

## OTHER:

2.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gaucher's disease is an inherited disorder caused by a mutation of the gene for enzyme glucocerebrosidase. The normal gene for this enzyme has been cloned by several laboratories. We have constructed high-titre, helper-free recombinant retroviruses containing this gene. We have shown that infection of cell lines from normal individuals and patients with Gaucher's disease with this retroviral vector results in increased glucocerebrosidase activity. The glucocerebrosidase gene has been transferred efficiently into progenitor cells and repopulating stem cell of mouse bone marrow and is expressed at the RNA and protein level in the progeny of CFU-S multipotential progenitor cells following gene transfer. The gene has also been transferred efficiently into human hematopoietic cell lines and progenitor cells, (CFU-GM and CFU-M). Production of vector derived RNA is seen in human hematopoietic cell lines of macrophage and other lineages and preliminary experiments indicate that vector derived glucocerebrosidase can raise enzyme activity to normal levels following transfer into hematopoietic progenitor cells from Gaucher patients. These experiments will help determine the feasibility of retroviral "gene therapy" for Gaucher disease.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02769-02 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Exploration of Strategies for the Treatment of AIDS

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|        |               |              |     |       |
|--------|---------------|--------------|-----|-------|
| PI:    | R. O. Brady   | Chief        | DMN | NINDS |
| OTHER: | R. R. O'Neill | Staff Fellow | DMN | NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Enzymology and Genetics, Neurochemical Methodology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.3

## PROFESSIONAL:

1.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two novel approaches for the treatment of AIDS will be examined. The first encompasses the development of anti-retroviral agents that traverse the blood-brain barrier. The second utilizes molecular biological approaches to inhibit virus replication.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02770-02 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Strategies for the Treatment of Autoimmune Neuropathies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |               |            |                   |       |
|---------|---------------|------------|-------------------|-------|
| PI:     | R. O. Brady   | Chief      | DMN               | NINDS |
| OTHERS: | R. H. Quarles | Chief      | LMCN              | NINDS |
|         | M. C. Dalakas | Unit Chief | MN                | NINDS |
|         | N. W. Barton  | Unit Chief | DMN               | NINDS |
|         | A. L. Weis    |            | Eastman Kodak Co. |       |

## COOPERATING UNITS (if any)

Laboratory of Molecular and Cellular Neurobiology  
Eastman Kodak Company Research Laboratories, Rochester, NY.

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Enzymology and Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.5

## OTHER

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of patients with peripheral neuropathy associated with benign monoclonal gammopathy have been shown to have circulating antibodies to specific glycoconjugates. The principle immunoreactive epitopes in a number of these individuals have been identified. It is proposed that immunoaffinity columns be prepared with appropriate ligands for selective apheresis trials to remove the reactive immunoglobulin from the patient's serum and to assess the effect of this procedure on the clinical course of the patients.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02771-02 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modification of Growth Factor Genes by Gene Targeting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Kulkarni

Sen. Staff Fellow

DMN

NINDS

OTHERS: S. Karlsson

Acting Section Chief

DMN

NINDS

L. Perry

Biologist

DMN

NINDS

## COOPERATING UNITS (if any)

Terry Fox Laboratory, Vancouver, CA. (K. Humphries)

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Molecular and Medical Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

12.0

## PROFESSIONAL:

11.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene targeting by homologous recombination can be used to activate or inactivate cellular genes in eukaryotic cells. The objective of this project is to alter the functional status of genes that control growth and maturation of specific tissues and study the biological consequences of these molecularly defined alterations. The interleukin 3 gene has been chosen for this study as it is a well characterized gene whose biological functions are well known. Activation of the interleukin 3 (IL-3) gene will be attempted in a cell line (32-D) which is IL-3 dependent for growth. Successful activation will therefore lead to a selective phenotype which will minimize screening and identification of the cells where the gene has been targeted to the correct location. Similarly, the biological importance of the IL-3 gene will be studied in the context of a whole organism by targeting a defective IL-3 gene to its cognate counterpart in embryonic stem cells by homologous recombination. These cells will subsequently be used to generate transgenic mice containing a defective IL-3 gene.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-02782-01 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Preparation of Transgenic Murine Analogs of Human Metabolic Storage Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |               |              |     |       |
|---------|---------------|--------------|-----|-------|
| PI:     | R. R. O'Neill | Staff Fellow | DMN | NINDS |
| OTHERS: | S. Karlsson   | Visiting     |     |       |
|         |               | Scientist    | DMN | NINDS |
|         | R. Brady      | Chief        | DMN | NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology

## SECTION

Enzymology and Genetics, Molecular and Medical Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

2.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Molecular genetic principles will be used to produce murine analogs of inherited human diseases for which spontaneous mutants are not available. Development of a transgenic mouse model of Gaucher's disease is a high priority aspect of this project.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS-02785-01 DMN

## PERIOD COVERED

October 1, 1988 to September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generation of mice with sickle cell anemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |   |                    |     |       |
|---------|---|--------------------|-----|-------|
| PI:     | S. Karlsson   | Acting Chief, M&MG | DMN | NINDS |
| OTHERS: | B. Dropulic   | Visiting Fellow    | DMN | NINDS |
|         | L. Perry  | Biologist          | DMN | NINDS |
|         | M. Dewey, Dept. of Biology, Univ. of S. Carolina            |                    |     |       |
|         | A. N. Schecter and C. Noguchi, Lab of Chem. Biology, NIAADK |                    |     |       |
|         | Y. Beugard, INSERM, Creteil, France                         |                    |     |       |

## COOPERATING UNITS (if any)

Laboratory of Chemical Biology, NIAADK  
Dept. of Biology, Univ. of S. Carolina  
INSERM, Creteil, France

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Molecular and Medical Genetics

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

1.0

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transgenic mouse technology can be utilized to produce animals expressing a foreign gene to high levels of its RNA and protein. The objective of this project is to generate mice that contain an abnormal beta globin gene (beta sickle Antilles). This gene has two mutations and generates sickle cell anemia in a heterozygous individual. High level expression of the gene is obtained by inserting the dominant control region from the human beta globin locus in cis to the Antilles gene. This dominant control region allows high level globin expression to occur. The human alpha globin gene is also inserted in cis in order to generate mice that can produce high levels of beta sickle Antilles and human alpha globins. After generating these mice, the transgenics will be mated to alpha thalassemic mice to eliminate the presence of mouse globins which disturb formation of sickle polymers.







ANNUAL REPORT

October 1, 1988 through September 31, 1989

Experimental Therapeutics Branch

National Institute of Neurological Disorders and Stroke

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## ANNUAL REPORT

October 1, 1988 through September 31, 1989

Experimental Therapeutics Branch  
Clinical Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke  
Thomas N. Chase, M.D., Chief

The goal of the Experimental Therapeutics Branch is to develop improved pharmacotherapies for neurologic disease. To this end the Branch operates a vertically integrated program of research extending from basic neurobiology to clinical trials. The focus of these investigative efforts remains on neurodegenerative disorders which impair motor and cognitive function.

The Branch is organized into four highly interactive operating components: 1) The Molecular Pharmacology Unit, directed since 1987 by Dr. David Sibley, conducts molecular and biochemical investigations to characterize central transmitter receptors and information transduction processes; 2) The Genetic Pharmacology Unit, until July of this year led by Dr. Donald Gelbert, seeks at the molecular level to develop pharmaceutical approaches to the selective regulation of gene expression within the mammalian central nervous system; 3) The Neurophysiologic Pharmacology Section, operating under the leadership of Dr. Judith Walters at the neuronal network level, studies basal ganglia function especially in relation to dopamine receptor mechanisms and the effect of drugs that influence motor behavior; and 4) The Clinical Pharmacology Section under Dr. Thomas Chase works clinically and in animal models to elucidate pathophysiologic mechanisms and evaluate novel pharmaceutical interventions.

### Genetic Pharmacology Unit

#### Pharmacology and Cellular Biology of Peptidergic Neurons

An increasing number of peptide transmitters has been identified in the brain over the last decade. The goal of the Unit is to develop an understanding of the basic regulatory mechanisms in cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic systems. The major thrust of the Unit over the past five years has been an examination of the regulation of neuropeptide gene expression using the pro-opiomelanocortin (POMC) gene as a model system for the study of other transmitter and transmitter-receptor gene regulation. Insight into the basic regulatory mechanisms at work in the POMC system will facilitate the application of molecular genetic approaches to the treatment of neurodegenerative disorders.



Since FY85 the Unit has utilized the AtT-20 tumor cell line to examine the intracellular regulation of POMC gene expression. Two approaches have been pursued: first, investigation of the linking steps between second messenger activation of protein kinases and cellular responses such as neuropeptide biosynthesis and secretion; and second, investigation of regulatory protein recognition sites on the upstream flanking region of the POMC gene. The first of these approaches has suggested that second messenger systems utilize both shared and distinct protein kinase substrates (possible third messengers) during propagation of intracellular signals. Since different second messenger activators produce analogous changes in POMC gene expression, the "focal" phosphoproteins (substrates for more than one protein kinase) were studied in detail in FY87-88. It was found that a 14 kDa phosphoprotein exhibited alterations in phosphorylation state that correlated with second messenger induced alterations in POMC gene expression. In addition, this protein was capable of translocating from the nucleus into the cytosol following phorbol ester stimulation, opening the possibility that it may serve as an intercompartmental signal transducer. The Unit is currently attempting to isolate and purify this protein.

In FY88, studies on the role of protein kinase C (PKC) in the induction of POMC gene transcription and  $\beta$ -endorphin secretion were conducted using the AtT-20 cell model. A technique was developed whereby AtT-20 cells could be rendered PKC deficient and ensuing effects on PKC and protein kinase A (PKA) dependent cellular processes were determined. It was found that 24 hour pretreatment of AtT-20 cells with synthetic diacylglycerol analogs effectively abolished PKC activity and PKC mediated  $\beta$ -endorphin secretion. In addition, this procedure also reduced corticotropin releasing hormone (CRH)-elicited  $\beta$ -endorphin secretion to a level attained by direct elevation of intracellular cAMP. This finding suggested a heretofore unknown role for PKC in CRF receptor linked signal transduction. The effects of rendering AtT-20 cells PKC deficient on CRH and second messenger induced POMC mRNA production were less pronounced, suggesting that secretory and biosynthetic pathways are not equally dependent on PKC.

In order to focus on neuropeptide gene transcription mechanisms, the Unit recently initiated methodologies designed to explore the role of the 5' promoter region of the POMC gene in the regulation of POMC transcription. In FY88, the 2 kilobase region of the murine POMC gene upstream from the POMC coding region was subcloned into a vector containing the bacterial lac operator. This construct was then utilized in an exonuclease protection assay to determine the locations on the POMC promoter of DNA sequences involved in binding of proteins which regulate POMC gene transcription. Initial experiments, conducted in FY88-89 have identified several protein binding sites in the -1000 to +1 region of the mouse POMC promoter. One of these sites, located approximately 115 base pairs upstream from the transcription start site, was homologous to a recently reported binding site for AP2 (a DNA binding protein thought to convey second messenger signals to transcriptional machinery).

In FY89, this putative AP2 site is being studied in detail, utilizing DNase I footprinting, and gel shift methodologies. Initial results have indicated that the putative AP2 binding site is a physiologically relevant site, capable of binding proteins residing in AtT-20 cell nuclei in a second messenger dependent manner. Continuing studies will attempt to discern the roles of individual second messenger systems and their interactions in the regulation of proteins binding to the AP2 site. In addition, an attempt will be made to isolate and characterize protein(s) binding to the AP2 site so that crucial studies examining the role of AP2 in the regulation of POMC gene transcription and the role of protein phosphorylation in this process can be conducted. These studies will further our understanding of the mechanisms involved in neuropeptide gene transcription and provide a model system to test future drug development schemes focusing on modulation of neurotransmitter biosynthesis.

#### Molecular Pharmacology Unit

The Molecular Pharmacology Unit continued investigating the biochemical, molecular, and pharmacological properties of dopamine and other neurotransmitter receptors in FY89. The long term goal of the Unit is the characterization of neurotransmitter receptor - mediated information transduction, and its regulation, across neuronal membranes. Dopamine receptors are used as representative model systems for the large class of neurotransmitter receptors which are linked to their biochemical effectors via guanine nucleotide binding regulatory (G) proteins. In order to characterize the D<sub>1</sub> and D<sub>2</sub> receptors at the biochemical and molecular levels and study their regulation, there are two major interrelated lines of research which are ongoing: 1) investigation of the cell biology, function and regulation of the receptors at the protein level; and 2) the molecular cloning of the receptor genes and investigation of gene structure and regulation in normal and pathophysiological states. The successful development of this latter project was a major focus of the Unit in FY89.

##### 1. Cell Biology and Regulation of Dopamine Receptors.

Our efforts in FY89 have been to capitalize on our FY88 discovery of various mammalian cell lines that express either D<sub>1</sub> or D<sub>2</sub> receptors and initiate studies of dopamine receptor regulatory mechanisms. For D<sub>1</sub> receptors we have been utilizing the NS20Y murine neuroblastoma cell line which expresses high levels of D<sub>1</sub> receptors. In FY89, we have shown that these D<sub>1</sub> receptors are functionally coupled to the Gs protein and the adenylate cyclase as demonstrated by GTP effects on agonist binding and pharmacologically specific agonist-induced cAMP generation in intact cell and membrane preparations. Furthermore, preliminary evidence in FY89 has indicated that acute exposure of these cells to dopamine results in a profound desensitization of the D<sub>1</sub> receptor-stimulated adenylate cyclase activity as well as a loss in D<sub>1</sub> receptor radioligand binding activity. We have thus established that the NS20Y cell line serves as an ideal model system to study agonist-mediated regulation of D<sub>1</sub> receptors coupled to adenylate cyclase activity. We are currently investigating the

underlying biochemical and molecular mechanisms associated with this process. We are also investigating the effects of chronic (days) agonist or antagonist exposure as well as evaluating the withdrawal periods to detect a potential supersensitization response. In addition, we are examining the effects of treating the cells with other agents such as steroid hormones to see if they may also modulate receptor activity.

For D<sub>2</sub> receptors, we have been characterizing the Y-79 human retinoblastoma cell line. The Y-79 cells exhibit particularly interesting characteristics as they exhibit few D<sub>2</sub> receptors under normal growth conditions but express extremely high quantities of D<sub>2</sub> receptors after being induced to differentiate with cAMP or butyric acid. The pharmacology of competition for [3H]-methylspiperone binding to differentiated Y-79 (dY-79) cell membranes by a series of dopaminergic antagonists verifies the D<sub>2</sub> receptor nature of this site exhibiting appropriate affinities and rank order of potencies. In addition, incubation with dopamine or other D<sub>2</sub>-selective agonists results in an approximate 50% reduction in forskolin-stimulated cAMP production in lysed dY-79 cells, which can be blocked by D<sub>2</sub>-selective antagonists. These data indicate that the D<sub>2</sub> receptors expressed in these cells are indeed functional. The ability to induce high levels of expression of functional D<sub>2</sub> dopamine receptors in a cultured human cell line should provide an excellent model system with which to examine the molecular events involved in the expression and regulation of this receptor system. We are currently evaluating these cells for the exhibition of agonist-induced desensitization or other regulatory phenomena for the D<sub>1</sub> receptors discussed above. In addition, we are using these cells as an in vitro system to evaluate the effects of drugs such as N-0437 and its resolved isomers on human D<sub>2</sub> receptors.

In FY89 we also completed development of an irreversible D<sub>2</sub> receptor antagonist. Previously, we synthesized N-(p-isothiocyanatophenethyl)spiperone (NIPS) a derivative of the high affinity D<sub>2</sub> selective antagonist, spiperone. The isothiocyanate moiety in this ligand renders it a potential affinity probe of the D<sub>2</sub> receptor. This property was evaluated in FY89. In vitro evidence was obtained using rat striatal membranes for an irreversible inhibition of D<sub>2</sub> receptors. Experiments in vivo indicated that NIPS can almost completely inactivate D<sub>2</sub> receptors without affecting D<sub>1</sub> dopamine receptors in the striatum or alpha<sub>1</sub>, alpha<sub>2</sub>, or muscarinic cholinergic receptors in the frontal cortex or 5-HT<sub>1A</sub> receptors in the hippocampus. These results suggest that NIPS is a highly selective irreversible inactivator of D<sub>2</sub> dopaminergic receptors and may prove useful in in vitro and in vivo functional studies of this receptor subtype. We are currently preparing to file an invention report on this ligand so that the Institute can decide whether or not to pursue patenting it.

In FY89 we synthesized a series of novel fluorescently-labeled ligands with high affinity and specificity for D<sub>1</sub> and D<sub>2</sub> dopamine receptors. The interaction of these fluorescent ligands with dopamine receptors was evaluated by examining their ability to compete for the binding of the radiolabeled antagonists [3H]-SCH 23390 or [3H]-



methylspiperone to rat striatal D<sub>1</sub> or D<sub>2</sub> dopamine receptors, respectively. In order to further evaluate the utility of these fluorescent probes for the cellular visualization of dopamine receptors, we have collaborated with Dr. Marjorie Ariano who has been most successful in demonstrating receptor-specific regional and cellular fluorescent labeling of D<sub>1</sub> and D<sub>2</sub> receptors in slices of rat forebrain, pituitary, retina, and superior cervical ganglion (SCG). The regional localizations of the dopamine receptors reflect previous work ascertained using *in vitro* receptor autoradiographic methods. The most robust regional localization of the D<sub>1</sub> receptor was found in the striatum and nucleus accumbens, displaying a membranous neuropil distribution. Medium sized neurons were the most prominent cell type that exhibited D<sub>1</sub> binding sites. Also, large cortical pyramidal neurons displayed this type of dopamine receptor population. The D<sub>2</sub> receptor was also prevalent within the striatum and nucleus accumbens, but did not show as much staining intensity as the D<sub>1</sub> receptor subtype. Medium sized and an occasional large neuron demonstrated fluorescent binding sites for D<sub>2</sub> receptors in these forebrain regions. We are currently using these probes, as well as additional probes under development, in simultaneous labeling experiments to ascertain whether or not D<sub>1</sub> and D<sub>2</sub> receptors can be shown to co-localize to the same neurons within the striatum and other brain regions.

## 2. Molecular Cloning of Dopamine Receptors.

In FY89 we constructed a rat striatal cDNA library in the lambda ZAP II vector for the purpose of cloning both D<sub>1</sub> and/or D<sub>2</sub> dopamine receptors. Our initial library screening strategy was to try to identify cDNA clones through low stringency cross-hybridization to short oligonucleotide or full length probes derived from a closely related protein. Until recently, the proteins most closely related to dopamine receptors which have been cloned and sequenced include the human beta<sub>1</sub>-, beta<sub>2</sub>-, and alpha<sub>2A</sub>-adrenergic receptors, the turkey beta<sub>1</sub>-adrenergic receptor, and the hamster and rat beta<sub>2</sub>-adrenergic receptors. Close inspection of the nucleotide sequences of the cDNAs or genes encoding these proteins reveals several regions of 21 bases or longer which are almost identical. We synthesized the corresponding oligonucleotide probes to these regions to be used in screening our cDNA library. While in the process of screening this cDNA library using a family of degenerate oligonucleotide probes, the sequence of a cDNA encoding a rat D<sub>2</sub> dopamine receptor was published. The group which isolated this cDNA employed a similar strategy as ours in that they used a restriction fragment of the hamster beta<sub>2</sub>-adrenergic receptor as a probe to screen, at low stringency, a rat brain cDNA library constructed from total brain RNA. Our own oligonucleotide probe approach was validated by the fact that one family of our probes was >90% homologous with the published sequence of the D<sub>2</sub> receptor cDNA and that this probe mixture was subsequently used by us to identify and isolate 5 cDNA clones from our rat striatal cDNA library which we now know to correspond to D<sub>2</sub> receptor cDNAs. These cDNAs are identical in sequence to each other, however, they differ from the published clone in that they have an additional 87bp sequence corresponding to an insertion of 29 amino acids in the region of the receptor believed to correspond to the third internal cytoplasmic loop.

We thus have identified and cloned what appears to be a splice variant of a rat D<sub>2</sub> dopamine receptor cDNA. In order to verify that this is indeed the case, we prepared an oligonucleotide probe to the insertion sequence as well as a probe to a common sequence and performed Northern analysis on rat striatal poly(A)<sup>+</sup> RNA. Both probes blotted an identical single band with equal intensity at 2.9 kb which corresponds to the D<sub>2</sub> receptor mRNA. The fact that both probes blotted the rat striatal D<sub>2</sub> receptor mRNA with equal intensity and that all of our clones isolated from the rat striatal cDNA library contain the insertion sequence, argues that the predominant isoform of the D<sub>2</sub> receptor in the striatum is the variant identified by us. The location of the published variant which was isolated from a total brain cDNA library is currently not known. We are presently performing Northern analysis with the consensus and insert probes on a number of different brain regions as well as other tissues such as pituitary, retina, and kidney to try to identify the regional distributions of these two D<sub>2</sub> receptor isoforms. It should be noted that this finding represents the first known example of alternative mRNA splicing giving rise to two different neurotransmitter receptor isoforms.

The functional significance of these two different D<sub>2</sub> receptor isoforms is presently not clear. It is interesting to note, however, that the difference occurs within the third large internal cytoplasmic loop. Mutagenesis studies using the beta<sub>2</sub>-adrenergic and the muscarinic receptors have indicated that it is this region of the receptors which is involved in coupling to G proteins. An intriguing hypothesis is that the two different D<sub>2</sub> receptor variants are thus coupled to different G proteins and different biochemical responses. In order to explore this further we are currently performing expression studies with the two different D<sub>2</sub> receptors using stable transfection of various mammalian cell lines. It should be noted that these constructed cell lines would be ideal model systems to screen drugs with in the development of new D<sub>2</sub> receptor ligands, particularly for agonists if there are, in fact, differential responses. We are thus in the process of preparing an invention report so that the Institute may proceed with filing a patent application on our splice variant of the D<sub>2</sub> receptor.

In FY89 we have examined the possibility that additional transduction pathways exist for D<sub>1</sub> receptors by using functional expression assays in *Xenopus* oocytes that have been injected with rat striatal poly(A)<sup>+</sup> RNA (mRNA). Defolliculated oocytes were assayed electrophysiologically 72 hr after injection with 50 ng of striatal mRNA. Application of 0.1 mM dopamine induced an inward current (40-100 nA) that was consistent with the activation of the endogenous oocyte Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channels. This current could also be elicited by the addition of the D<sub>1</sub>-selective agonist, SKF-38393 but not by the selective D<sub>2</sub> agonist, quinpirole. Prior addition of the dopamine antagonist cisflutixol completely abolished the dopamine-induced current but had no effect on the serotonin (5HT)-induced current which is also expressed from striatal mRNA. 0.1 mM dopamine was also found to stimulate 45Ca<sup>2+</sup> efflux by 2-3 fold in striatal mRNA-injected oocytes. This response was mimicked by SKF-38393 and blocked by the D<sub>1</sub>-selective antagonist, SCH-23390. No efflux was observed with 0.1 mM quinpirole and the dopamine



response was not blocked with 0.1mM of domperidone, a D<sub>2</sub>-selective antagonist. Assay of size-fractionated striatal mRNA using the oocyte/45Ca<sup>2+</sup> efflux assay revealed a single peak of dopamine-stimulated activity corresponding to an mRNA size of 2.5-3.0 kb. Oocytes which were injected with the peak mRNA fractions then prelabeled with myo[3H]inositol, additionally showed a 4-fold increase in IP<sub>3</sub> production in response to dopamine. In contrast, oocytes injected with the peak mRNA showed no elevation of cAMP levels in response to dopamine application. Moreover, exogenously applied db-cAMP did not elicit the electrophysiological or the 45Ca<sup>2+</sup> efflux response. These data thus indicate the existence of a D<sub>1</sub> dopamine receptor subtype which is coupled to phosphatidylinositol (PI) turnover and Ca<sup>2+</sup> mobilization in addition to a D<sub>1</sub> receptor subtype which activates adenylate cyclase activity.

In order to clone this novel D<sub>1</sub> receptor subtype coupled to PI turnover, we have constructed a cDNA library, using the size-fractionated rat striatal mRNA discussed above, in the lambda SWAJ-2 vector. This vector contains RNA polymerase sites flanking the polylinker and thus the cDNA insert region. It is thus possible to transcribe RNA from the cDNA off the vector and inject it into oocytes for translation and expression of the protein. The cloning strategy consists of dividing the cDNA library into a number of pools consisting of 10,000-100,000 clones each, running mRNA off each pool individually and injecting it into oocytes and assaying for a positive 45Ca<sup>2+</sup> efflux response. Once a positive pool is identified, it is subdivided into daughter pools and the process is repeated until a pure clone is obtained. This approach has been validated by the fact that it has been recently used to clone the serotonin 5HT<sub>1C</sub> and the substance K receptors, both of which are coupled to PI turnover and Ca<sup>2+</sup> mobilization.

#### Neurophysiological Pharmacology Section

Current focus in the Neurophysiological Pharmacology Section is on the basal ganglia and the potential for modulating basal ganglia function with drugs. In the past year, we have used neurophysiological techniques to explore unresolved issues relating to the roles of the different dopamine receptor subtypes in the substantia nigra and basal ganglia and the effects of dopamine on other neurotransmitter systems involved with processing information in this brain region. The dopamine system is critical to appropriate information processing in the basal ganglia. The role of the tonically active dopamine neurons appears predominately one of gain regulation, and may involve modulation of transmission mediated by other neurotransmitter systems. These cells do not appear to transmit specific phasic messages, but through relatively subtle changes in rate or firing pattern may induce changes in transmission and integration of information at postsynaptic sites. Dysfunction of this neuronal system has been implicated in the etiology of many neurological diseases, including Parkinson's disease, tardive dyskinesia, Huntington's chorea and torsion dystonia.

#### 1) D-1 and D-2 Dopamine Receptor Interactions.

Those dopamine receptors in the basal ganglia and substantia nigra which are located on cells postsynaptic to the dopamine neurons are often referred to as postsynaptic dopamine receptors. Our previous studies have led to a fundamentally new and exciting concept about the relative roles of the two receptor subtypes: they have shown that concurrent D-1 and D-2 receptor stimulation is necessary for full expression of postsynaptic receptor-mediated effects of dopamine and dopamine agonists in the basal ganglia. Studies investigating the location of the postsynaptic D-1 and D-2 receptors mediating the synergistic effects of D-1 and D-2 agonists on the activity of basal ganglia output neurons have indicated that the interaction between the two receptor subtypes cannot be localized to the globus pallidus or the substantia nigra. In addition, no evidence has been found to suggest that D-1 receptors within the substantia nigra pars reticulata are involved in mediating the synergistic effects on basal ganglia output elicited by systemic coadministration of D-1 and D-2 agonists. These results support the idea that the D-1 and D-2 receptors mediating the behavioral and neurophysiological effects of dopamine agonists are located in the striatum. However, it is currently unclear whether synergistic interactions between the two subtypes are intracellular or intercellular, i.e., whether the interacting receptors are located on the same cell or two different cells which interact in some manner.

In the past year, Dr. David Sibley of the Molecular Pharmacology Unit, ETB, has identified two neuroblastoma cell lines expressing D-1 receptors and two expressing D-2 receptors which we investigated in collaboration with Dr. Michael Rogawski (MNB) and Dr. Nancy Silva (LNP) using whole-cell patch clamp methods to determine whether these cultured tumor cell lines have membrane channels that are linked to the activation of these dopamine receptors. It was hoped that such a preparation might provide insight into the mechanisms underlying the function and possible interaction of these two receptor subtypes. However, the pressure ejection of D-1 selective agonists fenoldopam or D-2 selective agonists did not consistently modify sodium or potassium conductances in the voltage clamp mode in any of the tumor cells examined and did not modify resting membrane potential in a current-clamp mode or during current injection to evoke calcium spikes that were observed in some cells in current-clamp mode. It appears that the dopamine receptors on these tumor cell lines are not linked to the function of potassium, sodium or calcium channels. Plans are being made to investigate D-1/D-2 mechanisms in a striatal slice preparation in the coming year.

## 2) D-2 Autoreceptor/D-2 Postsynaptic Receptor Studies

Interest has focused on the question of whether the D-2 dopamine autoreceptors (those D-2 receptors located on the dopamine cell bodies) may constitute a distinct subset of receptors which could be selectively stimulated by a highly specific agonist. We have shown the existence of a greater receptor reserve at dopamine autoreceptor sites relative to postsynaptic dopamine receptor sites. This difference in spare receptor number at the two sites contributes to the interesting spectrum of effects of these partial dopamine agonists, as further supported by our

recent 2-deoxyglucose studies on these drugs. These agents may have some therapeutic advantages in the treatment of Parkinsonism and schizophrenia by virtue of their ability to reduce dopamine release and act as weak antagonists at normosensitive postsynaptic dopamine receptors while they may stimulate supersensitive postsynaptic dopamine receptors. The potential clinical usefulness of these partial dopamine agonists has been supported by clinical studies showing positive effects with one such drug, teguride, in patients with advanced Parkinsonism conducted by the Pharmacology Section of the ETB. Additional recent results have suggested that this mechanism of action may extend to some of the drugs acting through the serotonin system; we are currently exploring the hypothesis that certain drugs useful in the treatment of anxiety, such as buspirone, are partial agonists at 5HT<sub>1a</sub> receptors and that there is a relatively greater number of spare 5HT<sub>1a</sub> receptors at the autoreceptor sites in the serotonin system, as compared to postsynaptic sites, just as in the dopamine system.

### 3) Dopamine Receptor Mechanisms and Striatal Output.

Our studies and those of others have shown that the effects of dopamine on the basal ganglia are mediated in large part via the striatum. Anatomically, separate striatal efferents project from the striatum to the globus pallidus and the substantia nigra pars reticulata. The neurotransmitter contents of these systems are distinct. Although both projections contain GABA, different peptide neurotransmitters are found in these pathways. The striatopallidal fibers have a high concentration of enkephalin while the striatonigral fibers are enriched in dynorphin and substance P. We have utilized gamma-aminobutyric acid (GABA), GABA agonists and selective enkephalin agonists and antagonists to explore the ways in which the striatal neurons projecting to the globus pallidus and substantia nigra contribute to dopamine and dopamine agonist-induced changes in pallidal activity in these downstream areas. We have also investigated dopamine-receptor mediated processes in an animal model of Parkinsonism in which chronic dopamine denervation is achieved in rats with the use of 6-hydroxydopamine injections. Our neurophysiological results show that 6-hydroxydopamine lesion of the dopaminergic nigrostriatal pathway causes a functional downregulation of opioid and GABA receptors in the globus pallidus. We have also used quantitative receptor autoradiography to show decreases of 20 - 50% in mu and delta opioid receptors in the striatum, globus pallidus and substantia nigra pars reticulata of lesioned rats. These results suggest that dopamine normally has an inhibitory influence on striatopallidal GABAergic and enkephalinergic neurons and dopamine denervation causes increased activity of the predominantly inhibitory striatopallidal enkephalin and GABA containing neurons, inducing compensatory postsynaptic changes in the globus pallidus. We further observed that chronic opioid antagonist treatment reversed the 6-hydroxydopamine-induced subsensitivity to opioid-agonists observed in the globus pallidus. In addition, chronic naloxone treatment induced receptor upregulation of 75 - 200% in these areas in binding studies. The chronic opioid antagonist treatment did not reverse GABA receptor subsensitivity. The absence of a change in GABA receptor function in the presence of



opioid receptor changes points to a possible lack of interaction between the processes regulated by enkephalin and GABA receptors in the globus pallidus, at least at the level of the neurons postsynaptic to the striatopallidal input.

Studies on the effects of dopamine cell loss on the function of the striatonigral pathway indicate that the striatonigral pathway is affected by lesion of the dopamine cells in a manner quite different from the striatopallidal pathway. A marked increase in sensitivity to GABA is observed in the substantia nigra in the 6-hydroxydopamine-lesioned rats. These observations imply that tonic GABA release in the substantia nigra pars reticulata, presumably from striatonigral terminals, is reduced by 6-hydroxydopamine lesion of the nigrostriatal dopamine pathway, and that dopamine may have a net excitatory effect on the nigrostriatal pathway in normal rats. The results from 2-deoxyglucose studies are consistent with our hypothesis that the dopamine agonists, especially those stimulating D-1 dopamine receptors, exert an apparent excitatory effect on the striatonigral pathway in 6-hydroxydopamine-treated rats. The results do not support a correlation between increased glucose utilization or inhibition of substantia nigra pars reticulata activity and turning behavior: glucose utilization increases observed in awake behaving rats following D-1 stimulation likely represent a primary drug effect and not a consequence of movement or sensory feedback. We hypothesize that the increased glucose utilization is associated with increased activity in the GABAergic striatonigral pathway in the lesioned animals as predicted by other experimental approaches.

An issue of considerable interest to the clinical investigations of the Branch is the mechanism underlying the development of motor fluctuations and the alterations in the thresholds for the therapeutic and dyskinetic effects of l-dopa and the strategies for treating this phenomena. The intermittence of the l-dopa treatment has emerged as an important issue in this regard. To gain further insight into this issue, we have examined the effects of chronic levodopa treatment in 6-hydroxydopamine lesioned rats on the sensitivity of neurons in the substantia nigra pars reticulata to systemic SKF 38393 administration and GABA iontophoresis. The results demonstrated that twice daily injections of l-dopa for 19 days abolishes the effects of SKF 38393 on substantia nigra pars reticulata activity just as it reduced the D-1 agonist-induced rotation. Concomitant with this change, chronic levodopa injection reversed the lesion-induced supersensitivity of substantia nigra pars reticulata neurons to iontophored GABA. Neither of these effects were produced by continuous infusion a l-dopa by osmotic pump for 19 days, a procedure that produces average daily blood levodopa levels similar to those produced by chronic levodopa injection. These results suggest that large variations in blood levodopa levels may lead to desensitization of D-1 and GABA receptor mediated mechanisms of basal ganglia output through the substantia nigra pars reticulata which had been sensitized by processes resulting from the loss of dopamine input to the striatum. They provide additional support for the idea that GABA-related phenomena underlie the response of the substantia nigra pars reticulata to D-1 dopamine agonists. It would appear likely that a similar mechanism

underlies the desensitization of the behavioral responses to D-1 dopamine agonist administration induced by the same l-dopa treatment. Finally, these results are relevant to clinical concerns regarding optimal l-dopa therapy because they imply that the time course of the changes in blood levels of levodopa has effect on mechanisms mediating the response to this therapy.

#### 4) Role of the Pedunculopontine Tegmental Nucleus

The pedunculopontine tegmental nucleus is a relatively unexplored region only recently emerging as an area potentially significant in regard to basal ganglia function. Neuroanatomical tracing studies have identified extensive connections between the pedunculopontine tegmental nucleus and the basal ganglia and substantia nigra. We have previously shown that kainic acid induced lesions of the pedunculopontine tegmental nucleus cause marked reductions in the number of spontaneously active dopamine cells in the substantia nigra of the lesioned animals. Investigation of the mechanism underlying this phenomena and the time course of its development suggest that at 7 days after the lesion, the silent dopamine neurons are in a state of depolarization inactivation, resembling the condition seen after chronic neuroleptic treatment. Studies suggest the dopamine cell inactivation is not a consequence of an early excitation induced by stimulation of the pedunculopontine tegmental nucleus cells by kainic acid which is then sustained by compensatory mechanisms because it results in a depolarization inactivation-induced decrease in dopamine release. Rather, the depolarization inactivation of the dopamine neurons appears due to the kainic acid-induced destruction of the pedunculopontine tegmental nucleus and subsequent alterations of neuronal influences on the substantia nigra pars compacta dopamine system. Thus, the pedunculopontine tegmental nucleus appears to exert a very significant tonic influence on substantia nigra dopamine cell activity. This apparently profound influence of the pedunculopontine tegmental nucleus over the substantia nigra pars compacta is even more remarkable in light of the fact that no other afferent of the region has been shown to have such an effect.

#### Clinical Pharmacology Section

Research conducted by the Clinical Pharmacology Section links basic neuroscience investigations carried out by other Branch components with the neurologically disordered patient. Clinical and preclinical studies apply transmitter pharmacologic approaches to the development of improved symptomatic therapies. In addition, pathogenetic mechanisms are studied that might provide a basis for more definitive pharmaceutical interventions. These investigative efforts remain primarily focused on Parkinson's disease and related extrapyramidal disorders and to a lesser extent on Alzheimer's disease and related degenerative dementias.



## Parkinson's disease

Central pathogenetic mechanisms for the motor response complications occurring in levodopa treated patients with advanced Parkinson's disease have been intensively investigated during the past year. One clinical study sought to evaluate the contribution of dopaminergic mechanisms at the presynaptic level. The duration of the antiparkinsonian action of levodopa was measured in patients with various response patterns to the oral administration of the dopamine precursor. Deterioration in motor scores after abrupt cessation of a steady-state intravenous levodopa infusion followed two successive rates: an initial rapid phase, followed by a terminal slower phase. The initial decay slope appeared to be an accurate index of processes regulating the duration of the antiparkinsonian action of levodopa, clearly differentiating the four levodopa response groups studied: 1) patients never before treated with levodopa; 2) those with mild parkinsonism who still enjoy an uncomplicated response to dopamine precursor therapy; 3) patients with wearing-off phenomenon, and finally 4) those with on-off fluctuations and severe dyskinesias. The initial decay slope correlated closely with the total severity of motor fluctuations occurring with standard oral levodopa therapy. The antiparkinsonian efficacy half-time exceeded plasma levodopa half-life in the two nonfluctuating groups, approximated it in patients with wearing-off responses, and was significantly shorter in those with on-off fluctuations. The increasing rates at which the antiparkinsonian response decays with advancing disease appears to account for wearing-off fluctuations and reflects the loss of natural sites for dopamine storage and release which compromises the brain's ability to buffer swings in levodopa availability occurring as a consequence of the drug's periodic oral administration.

Degeneration of dopamine terminals may expose dopamine receptors in patients receiving oral levodopa to nonphysiologic oscillations in transmitter levels. To evaluate the hypothesis that resultant postsynaptic receptor alterations contribute to the appearance of on-off phenomena and peak dose dyskinesias, we compared the acute dose-response relationship of levodopa in parkinsonian patients evidencing various responses to standard oral levodopa. While the threshold dose for an antiparkinsonian effect did not change, the threshold for dyskinesia induction showed a progressive reduction when levodopa naive patients, those with a stable response to oral administration, and those with wearing-off or on-off fluctuations were compared. Concomitantly, the therapeutic window for levodopa narrowed and the levodopa dose-antiparkinsonian response slope increased. The antiparkinsonian threshold dose correlated best with indices of natural disease progression, while the dyskinesia threshold dose, therapeutic window, and dose-response slope related most closely with drug treatment parameters. Since the acute effects of levodopa injections largely reflect the preferential release and thus the postsynaptic availability of newly synthesized dopamine, our results implicate postsynaptic receptor changes in the pathogenesis of motor response complications: the steepening of the dose-response relationship for levodopa favors the development of on-

off fluctuations while the diminished threshold for abnormal involuntary movements favors the appearance of peak dose dyskinesias.

A related clinical study explored the possibility that different pharmacologic mechanisms underlie the ability of levodopa to both ameliorate parkinsonian signs and induce abnormal involuntary movements. The duration of the dyskinetic and antiparkinsonian actions of levodopa and its dose-response profile for both these motor effects was evaluated in parkinsonian patients at various stages of disease. We found the duration of the dyskinetic action of levodopa to be significantly shorter than the duration of its antiparkinsonian effect. Moreover, the decay curve for dyskinesia was curvilinear and best described by a quadratic function, while that for parkinsonism was linear. We also observed that the minimum intravenous dose of levodopa required to produce abnormal involuntary movements was significantly lower in the two levodopa response groups with fluctuations than in the two groups with no response instability. In contrast, the smallest dose necessary for a minimally detectable improvement in parkinsonian signs did not differ significantly among the four groups. The demonstration of different pharmacological profiles for dyskinetic and parkinsonian signs could have important implications for the design of improved approaches to the symptomatic treatment of Parkinson's disease.

As a test of the value of continuous stimulation of dopamine receptors in the management of parkinsonian motor complications, we examined the effects of round-the-clock levodopa infusions on clinical state and central dopaminergic mechanisms. Motor fluctuations gradually improved during the 7-12 days of continuous therapy; motor variance scores ultimately decreased by nearly 50%. Dose-response parameters also changed significantly in response to the stable-dose levodopa infusion: the therapeutic window increased, mainly as a result of the increase in dyskinesia threshold; the dose-response curve shifted to the right; and the duration of the antiparkinsonian action of levodopa lengthened. Motor fluctuations began to worsen again about 6 days after resumption of standard oral levodopa treatment. These results support the view that intermittent levodopa replacement therapy modifies the responsiveness of dopaminoreceptive cells, most likely at the receptor level, and that these changes may be reversed by steady-state levodopa administration. It would appear that continuous dopaminomimetic therapies may have prophylactic as well as palliative value in patients with advanced Parkinson's disease.

A preclinical study of the biochemical and behavioral effects of chronic levodopa administration has provided new insight into the pathogenesis of motor response complications. To evaluate whether the constancy of dopamine receptor stimulation influences subsequent pharmacologic responses, the effect of continuous and intermittent levodopa administration were compared in an animal model of Parkinson's disease. Initial results indicate that apomorphine-induced contralateral rotation in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway are considerably enhanced in animals treated for 30 days with intermittent levodopa compared to those receiving the same

daily dose of levodopa continuously or to saline treated controls. Dopamine receptor up-regulation in the denervated striatum relative to the intact striatum was statistically significant for D-1 but not D-2 receptors; this asymmetry in dopamine receptor levels was diminished following intermittent levodopa treatment. The intermittent nature of levodopa administration appears more important than the presence of levodopa itself in the development of behavioral hypersensitivity.

The development of highly selective agonists and antagonists for both the D-1 and D-2 dopamine receptor subtypes has greatly facilitated studies of their localization and function. We applied the quantitative technique of receptor autoradiography, using the recently introduced iodinated ligand, [125I]SCH 23982, to quantify D-1 dopamine receptors in several discrete areas of rat brain. The binding of the highly selective D-1 receptor ligand to preparations of prefrontal cortex was saturable, specific and of high affinity. Although [125I]SCH 23982 was found to predominantly label D-1 receptors, a small fraction of binding was to serotonin receptors. D-1 receptors were observed throughout the forebrain, especially in substantia nigra pars reticulata, nucleus accumbens, corpus striatum, and entopeduncular nucleus. Previous reports dealing with the distribution of D-1 receptors were based on the use of tritium labelled ligands. But these yield only semiquantitative results in contrast to iodine-125 labelled compounds which provide a much more accurate quantitative estimate of receptor density.

Preclinical neurophysiologic and behavioral evidence previously reported by the Section suggested that concurrent activation of both D-1 and D-2 dopamine receptors may be necessary for the full expression of functions classically linked to the dopamine system. Although synergy between selective D-1 and D-2 receptor agonists in the induction of DA mediated behaviors has been established, less is known about the behavioral interactions between selective D-1 and D-2 receptor antagonists. The cataleptogenic properties of the selective D-1 antagonist SCH 23390 and the selective D-2 antagonist raclopride were thus compared. Both SCH 23390 and raclopride induced catalepsy in a dose-dependent manner. When SCH 23390 was administered in combination with increasing doses of raclopride, the dose-response curve for raclopride was shifted 10 times to the left. The combination produced significantly more intense catalepsy than raclopride or SCH 23390 alone. These findings suggest that mixed D-1/D-2 antagonists possess higher antipsychotic potential than drugs acting mainly on only one dopamine receptor subtype, and that the addition of a selective D-1 antagonist like SCH 23390 might allow a decrease in the clinically effective dose of neuroleptics acting predominantly on D-2 receptors.

The relative contribution of D-1 and D-2 receptor mediated mechanisms to the behavioral changes that follow chronic dopamine agonist exposure was examined in a related preclinical study. Selective D-1 and D-2 dopamine agonists SKF 38393 and LY 171555 were administered to intact rats for three weeks. Treatment with the D-1 agonist induced behavioral supersensitivity in response to apomorphine, while treatment with the D-2 agonist resulted in a subsensitive behavioral response. Apomorphine-



induced stereotypic behaviors were augmented by chronic treatment with the combination of these drugs, but were different in nature from those observed in animals treated with SKF 38393 alone. The behavioral response to LY 171555 was affected only by chronic treatment with the combination of selective agonists, while chronic SKF 38393 treatment resulted in an enhanced behavioral response to SKF 38393 itself. Long-term D-1 receptor stimulation may thus be both necessary and sufficient for the development of dopamine agonist-induced behavioral sensitization in the intact rat. Moreover, it would appear that a synergistic interaction between receptor subtypes may underlie certain behavioral effects of chronic agonist exposure.

A clinical study sought to evaluate the contribution of D-1 dopamine receptor mediated mechanisms to the motor changes associated with various hyperkinetic extrapyramidal diseases. SKF 38393, the most selective D-1 receptor agonist currently available for human administration, was given to patients with various extrapyramidal disorders. Subacute treatment, over a wide range of doses, had no clinically significant effect on motor function in patients with tardive dyskinesia or idiopathic torsion dystonia, or on either motor or cognitive function in patients with Huntington's disease or Tourette syndrome. Since biochemical and endocrinological results indicated the drug crossed the blood brain barrier in pharmacologically significant amounts, the lack of response to SKF 38393 could reflect a very limited role for D-1 receptor mediated mechanisms in hyperkinetic extrapyramidal motor disease. More likely, however, the inability of SKF 38393 to modify motor function in these extrapyramidal disorders reflects methodological limitations such as the fact that the drug is only a partial D-1 receptor agonist.

#### Alzheimer's disease

Studies of the relation between transmitter system abnormalities and cognitive function deficits as well as investigations of the pathogenesis of selective neurodegenerative processes have made substantial progress during the past year. One of the most consistent cerebral abnormalities occurring in patients with Alzheimer's disease is the loss of cortical cholinergic terminals due to degeneration of projections from the nucleus basalis. Nevertheless, efforts to ameliorate symptoms through the administration of cholinomimetics have thus far yielded no clinically significant benefit. Conceivably, these generally negative therapeutic results reflect a concomitant loss of cholinceptive cells in cortical projection areas. An evaluation of this possibility, now being conducted by means of single-photon emission computed tomography (SPECT) scanning following administration of iodine 123-labeled 3-quinuclidinyl-4-iodobenzilate (123I-QNB), suggests substantial decrements in apparent QNB binding occurs in Alzheimer's disease, especially in parietotemporal areas we have previously reported to be maximally hypofunctional in relation to glucose metabolic rates.

Progressive supranuclear palsy (PSP) is also associated with a relatively selective loss of acetylcholine containing neurons. Since cortical cholinceptive neurons appear relatively preserved, it is

possible that pharmacologic stimulation of the cholinergic innominate-cortical and septo-hippocampal pathways might benefit cognitively impaired PSP patients. Although orally administered physostigmine appeared to produce an inverted U-shaped dose-response curve with respect to effects on verbal memory in an initial dose-finding phase, a 10-day cross-over trial at the previously determined best dose produced no consistent change in short or long term memory. Drug treatment also had no significant effect on extrapyramidal motor function, nor did cerebrospinal fluid acetylcholinesterase activity change. These results approximate those obtained in Alzheimer's disease and suggest that the marginal antidementia efficacy of physostigmine may reflect the relatively low central pharmacologic activity attained at customary oral dose levels.

Cortical norepinephrine levels are often reduced in Alzheimer's disease presumably due to degeneration of rostral projections from the locus ceruleus. In view of the relative preservation of cortical postsynaptic noradrenergic receptors as well as the reported ability of adrenergic agonists to ameliorate memory loss in the experimental animal, clinical trials have been completed with two centrally active alpha-2 adrenoceptor agonists. In the first study, clonidine was found to produce no consistent alteration in any of several tests of attention, or of verbal or visuospatial memory. This failure may have been attributable to the appearance of dose limiting side effects, especially sedation and hypotension. A subsequent clinical trial of an even more selective alpha-2 adrenergic agonist, guanfacine, with purportedly less sedative effects, was thus undertaken. During an initial dose-finding phase or the subsequent best-dose guanfacine comparison with placebo, conducted at the highest dose free of adverse effects, none of the neuropsychological measures employed evidenced any consistent change. These results indicate that maximum tolerated doses of alpha-2 adrenergic agonists alone confer no therapeutic benefit to patients with Alzheimer's disease.

Episodic memory has been linked to dopamine function in healthy individuals and appears to be selectively impaired in untreated parkinsonian patients. Last year we reported that dopaminergic treatment benefitted effortful episodic memory in those with Parkinson's disease. However, improvements were relatively selective and generally modest. To further evaluate the contribution of dopamine mediated synaptic function to human cognitive function, the acquisition and retrieval of verbal and visuospatial memory tasks were studied in parkinsonian patients both while receiving levodopa/carbidopa ("on") and when the medication's antiparkinsonian effect had worn off ("off"). Tests of immediate episodic memory failed to demonstrate any consistent relation between the patients' motor response status ("on" compared with "off") and performance accuracy. Significant differences did, however, emerge in tests of delayed recall of complex verbal materials: both paired associate learning and logical memory tests were slightly, yet consistently, improved during the levodopa induced "on" state. The profound changes in motor function induced in parkinsonian patients by levodopa thus appear to have a modest, albeit consistent, analog in the cognitive realm.



Patients with Alzheimer's disease evidence cortical somatostatin reductions, especially in cerebral areas most abnormal on PET/FDG scans; we have also reported that spinal fluid concentrations of this neuropeptide are decreased in proportion to the severity of cognitive dysfunction. Since these observations suggest that a loss of cortical somatostatin-containing interneurons might contribute both to the cortical hypometabolism and the intellectual decline found in Alzheimer's disease, a clinical trial is now in progress to test the antidementia efficacy of SMS 201-995, a synthetic analog of somatostatin. This synthetic octapeptide has a much higher potency and a substantially longer biological half-life than naturally occurring somatostatin. To date Alzheimer patients have received SMS 201-995 either by subcutaneous injection or intravenous infusion with no consistent evidence of cognitive improvement. Adverse effects have been limited to mild, transient abdominal cramps and hyperglycemia.

Endogenous, neurotoxic excitatory amino acids have been implicated as possible contributors to the pathogenetic cascade in Alzheimer's disease. Of the various glutamate receptor subtypes studied, the N-methyl-D-aspartate (NMDA) complex appears most affected. To evaluate NMDA receptor alterations, postmortem tissues from patients with Alzheimer's disease were compared to age-matched controls for MK-801 binding. We found the density of [3H]MK-801 binding sites to be highest in the temporal pole of both Alzheimer and control brains. There were no consistent differences between patients and controls in binding density in any of the areas studied. Kd values in parietal association cortex were significantly higher than affinity constants in all other regions; the hippocampus had the lowest affinity constant. There was a close positive correlation between symptom duration and number of hippocampal binding sites. Age at death correlated negatively with [3H]MK-801 binding sites in control subjects in frontal lobe and hippocampus, but positively in Alzheimer cases in occipital lobe and hippocampus. In Alzheimer's disease, glutamatergic terminals may be lost faster than NMDA receptor-bearing neurons, resulting in denervation supersensitivity; in normal individuals, the opposite may occur, accounting for the negative correlation between age at death and Bmax values. Our results do not exclude the possibility that the presence of NMDA receptors is a necessary, but hardly sufficient, basis for cell death and are consistent with other biochemical data suggesting a relatively high degree of regional and neuronal selectivity.

Test of the excitotoxin hypothesis for Alzheimer's disease ultimately depends on the availability of drugs which act safely within the human central nervous system to block NMDA receptors. In a laboratory study completed during the past year we compared the neuroprotective activity of the non-competitive NMDA antagonist dextromethorphan (DM), a widely used non-prescription cough suppressant, with MK-801, a toxic yet potent excitotoxin antagonist. While MK-801 significantly reduced the depletion of striatal choline acetyltransferase activity caused by quinolinic acid, DM at highest tolerated doses, was unable to protect against excitotoxic damage. This failure most likely reflects the drug's relatively weak NMDA receptor blocking activity. At

the doses tested, DM may not achieve a concentration in brain sufficient to prevent excitotoxic damage resulting from the acute intracerebral injection of an NMDA agonist.

A related study sought to assess central pharmacologic mechanisms for potential excitotoxin antagonists, particularly MK-801, which act as functional NMDA antagonists by stimulating intrachannel phencyclidine (PCP) sites. To this end we evaluated the effects of PCP on cerebral glucose utilization using quantitative autoradiography. PCP was found to markedly elevate cerebral metabolism in selected cortical areas, most notably in the visual, entorhinal and limbic areas. Portions of thalamus, striatum, globus pallidus and substantia nigra were also significantly activated. PCP clearly exerts a selective effect on brain glucose metabolism and thus presumably on neuronal function in areas linked to cognitive and extrapyramidal motor function. Metaphit, a PCP receptor acylating agent developed in this laboratory, blocked most of these PCP actions. When administered alone, metaphit reduced glucose utilization rather uniformly throughout the brain. The ubiquitous presence of glutamatergic synapses in the central nervous system would be consistent with glucose utilization changes of the magnitude and extent observed.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02578-07 ET

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology and Cellular Biology of Peptidergic Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Donald R. Gehlert, Ph.D., Acting Head, Genetic Pharmacology Unit, ETB, DIR, NINDS.

M. Rinaudo, M.D. Visiting Fellow, Katherine Conant, M.D., Staff Fellow, Experimental Therapeutics Branch, NINDS

## COOPERATING UNITS (if any)

G.D. Searle and Company, CNS Division; Human Genetics Branch, NICHD

## LAB/BRANCH

Experimental Therapeutics Branch

## SECTION

Genetic Pharmacology Unit

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892.

## TOTAL MAN-YEARS:

4

## PROFESSIONAL:

2

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In FY 89, the Genetic Pharmacology Unit concentrated on two projects: 1) The elucidation of signal transduction mechanisms involved in proopiomelanocortin (POMC) gene expression, and 2) Investigation of cis-acting elements in the 5' flanking region of the POMC gene

## 1) SIGNAL TRANSDUCTION MECHANISMS INVOLVED IN POMC GENE EXPRESSION

Past investigations conducted in this Unit, utilizing the AtT-20 tumor cell model, indicated that regulation of POMC gene expression occurs primarily at the transcriptional level in response to activation of the cAMP second messenger system by CRF. However, studies conducted in FY 88 suggested that the effects of CRF receptor activation on endorphin secretion may also be dependent on the diacylglycerol-protein kinase C system and experiments performed in FY 89 have supported this suggestion. Since, POMC gene transcription is elevated by activation of either cyclic AMP-dependant protein kinase (via cAMP) or protein kinase C (via diacylglycerol analogs), we are currently extending studies of a novel phosphoprotein (pp14) which is a substrate for both of these kinases and is also capable of shuttling from the nuclear to the cytosolic compartment in AtT-20 cells in response to activation of either kinase. Initial direct microsequencing procedures have failed to produce reliable sequence information for pp14, necessitating the utilization of traditional chromatographic procedures which are in progress.

## 2) CIS-ACTING ELEMENTS IN THE 5' FLANKING REGION OF THE POMC GENE

Experiments begun in FY 88 concerning the locations of putative cis-acting elements in the 5' flanking region of the POMC gene have been continued in FY 89. Several exonuclease "stop sites", have been demonstrated, suggesting the existence of several protein binding sites. In particular, a putative binding site for AP2 (Activator Protein 2), a recently characterized transcription factor capable of linking second messenger signals to gene transcription machinery, was discovered. Current studies are focusing on: 1) the identity of the protein(s) which bind to the POMC gene AP2 binding site, and 2) the mechanism whereby changes in second messenger activities in the cells can modulate POMC gene expression via AP2.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02263-13 ET

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Pharmacological Studies of Dopamine Receptors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Sibley, Ph.D., Head, Molecular Pharmacology Unit, Experimental Therapeutics Branch, NINDS

Frederick J. Monsma, Jr., Ph.D., IRTA Fellow; Loris D. McVittie, Ph.D., IRTA Fellow; Anne C. Barton, Ph.D., IRTA Fellow; Yasuyuki Hatada, M.D., Ph.D., Visiting Fellow; Mario Rinaudo, M.D., Visiting Fellow, Experimental Therapeutics Branch, NINDS

## COOPERATING UNITS (if any)

Lab. Cell Biology, NIMH; Dept. Anatomy & Neurobiology, U Vermont; Center for Molecular & Behavioral Neurosciences, Rutgers U; Molecular Probes, Inc.; American Type Culture Collection

## LAB/BRANCH

Experimental Therapeutics Branch, CNP

## SECTION

Molecular Pharmacology Unit

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

4.5

## PROFESSIONAL:

4.5

## OTHER:

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## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long-term goal of this project is the biochemical and molecular characterization of dopaminergic receptor mediated information transduction, and its regulation, across neuronal membranes. Two major interrelated areas of research on D-1 and D-2 dopamine receptors are currently under investigation.

1. Cell biology and regulation of dopamine receptors. The ligand binding and functional properties of D-1 and D-2 dopamine receptors on various neuroblastoma and retinoblastoma cell lines were characterized. The D-1 receptors were shown to be functionally coupled to the stimulation of adenylate cyclase activity while the D-2 receptors were shown to inhibit this enzyme. These cell lines were further investigated as model systems for the study of dopamine receptor regulatory events. Agonist-induced desensitization and down regulation of the D-1 dopamine receptors was demonstrated. A variety of novel fluorescent and affinity ligands for dopamine receptors were synthesized and evaluated. One affinity ligand was shown to exhibit irreversible binding properties both *in vitro* and *in vivo* to D-2 but not other receptors. Several fluorescent ligands were used to anatomically localize dopamine receptors at the cellular level within brain slices and other tissues. D-1 receptors were localized to most of the cells in the striatum as well as numerous fibers while the D-2 receptors appeared to be confined to a smaller population of neurons.

2. Molecular cloning of dopamine receptors. A rat striatal cDNA library was constructed and screened with oligonucleotide probes to potentially conserved regions among catecholamine receptors. Several identical clones were isolated and determined to represent a splice variant of the rat D-2 dopamine receptor. This finding represents the first known example of alternative mRNA splicing giving rise to two different neurotransmitter receptor isoforms. The neuroanatomical localization and functional significance of these two different D-2 receptor isoforms is currently under investigations. A novel subtype of the D-1 receptor was discovered by expressing rat striatal mRNA in *Xenopus* oocytes. This new D-1 subtype is linked to the stimulation of phosphatidylinositol turnover and mobilization of calcium. Efforts to clone this receptor using the oocyte expression system are underway.

20-ET/IR



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02139-15 ET

## PERIOD COVERED

October 1, 1988 through September 31, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith R. Walters, Ph.D., Chief, Neurophysiological Pharmacology Section, Experimental Therapeutics Branch, NINDS

Debra Bergstrom, Ph.D., Barton Weick, D.V.M., Ph.D., Helen Pan, Ph.D., Marianne Beninato, Ph.D., Experimental Therapeutics Branch, NINDS

Joanne Carlson, Ph.D., Dept. Psychiatry, School of Medicine, University of California San Diego

## COOPERATING UNITS (if any)

Clinical Pharmacology Section, Experimental Therapeutics Branch; Nuclear Medicine Department, Clinical Center, NIH; University of Virginia

## LAB/BRANCH

Experimental Therapeutics Branch, CNP

## SECTION

Neurophysiological Pharmacology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

6.1

## PROFESSIONAL:

5.1

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1) Roles of D-1 and D-2 Dopamine Receptors in Basal Ganglia. Contrary to suggestions in the literature, we find no evidence that D-1 receptors within the substantia nigra pars reticulata are involved in mediating the effects of concurrent D-1 and D-2 receptor stimulation which we have shown necessary for full expression of postsynaptic receptor-mediated effects of dopamine and dopamine agonists in the basal ganglia; local interactions within the striatum appear likely.

2) D-2 Autoreceptor / D-2 Postsynaptic Receptor Studies. Due to the existence of a greater D-2 receptor reserve at dopamine autoreceptor sites relative to postsynaptic dopamine receptor sites, agents which are partial agonists at D-2 receptors exhibit recently appreciated spectrum of effects which may be therapeutically useful. Similar mechanisms may underlie significant drug effects at 5HT<sub>1a</sub> sites.

3) Consequences of Dopamine Cell Degeneration in the Basal Ganglia. In an animal model of Parkinsonism, we have found neurophysiological evidence that intermittence of L-dopa treatment is a significant variable in determining degree of desensitization of D-1 and GABA receptor mediated processes in the substantia nigra. Neurophysiological evidence for dopamine lesion-induced decreases and D-1 receptor induced increases in activity of GABAergic striatonigral pathway is supported by 2-deoxyglucose studies. Dopamine lesion-induced down-regulation of opioid receptors but not GABA receptors in the globus pallidus is functionally reversed by opiate antagonist treatment.

4) Role of the Pedunculopontine Tegmental Nucleus. The pedunculopontine tegmental nucleus (PPN) exerts a very significant tonic influence on substantia nigra dopamine cell activity as demonstrated by the marked reduction in the number of spontaneously active dopamine cells in rats following kainic acid lesions on the PPN. The reduction appears due to depolarization inactivation of the dopamine neurons caused by loss of the PPN and subsequent alterations in neuronal influences on the substantia nigra pars compacta dopamine system.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02265-13 ET

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology, Biochemistry and Physiology of Central Neurotransmitters

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas N. Chase, M.D., Chief, Clinical Pharmacology Section, Experimental Therapeutics Branch, NINDS

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## COOPERATING UNITS (if any)

NIMH Clinical Brain Disorders Br.; Dept. Psychiatry, U. Maryland; Tissue Research Center, Harvard U., Dept. of Psychiatry, Utah School of Medicine, Lab. de Medicine, Experimentale Hopital de la Salpetriere

## LAB/BRANCH

Experimental Therapeutics Branch

## SECTION

Clinical Pharmacology Section, CNP

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

11

## PROFESSIONAL:

9

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to develop improved pharmacotherapies for selected neurodegenerative disorders, especially Parkinson's disease and Alzheimer's disease.

In Parkinson's disease, wearing-off phenomenon occurring with levodopa therapy appears to reflect presynaptic dopamine neuron degeneration, occurring as a consequence of natural disease progression. In contrast, peak dose dyskinesias and on-off fluctuations seem to reflect alterations in the dyskinesia threshold and dose-response relation for levodopa resulting from secondary post junctional alterations, occurring as a result of chronic intermittent stimulation. The latter changes may be plastic, since all neuropharmacologic indices tend to normalize and motor complications tend to remit with continuous levodopa replacement. Results from an animal model of Parkinson's disease provides further support for the view that the intermittent nature of levodopa administration is more important than the presence of levodopa itself in the development of the post synaptic changes in the dopamine system.

In Alzheimer's disease, substantial decrements in cerebral muscarinic receptor binding, especially in parietotemporal cortex, were found on SPECT scanning. Other clinical results indicate that the marginal therapeutic effects of cholinomimetics such as physostigmine might also reflect the attainment of inadequate central pharmacologic activity at customary dose levels. Receptor binding studies with [3H] MK-801 in postmortem tissues from Alzheimer patients suggest glutamatergic terminals may be lost faster than NMDA receptor-bearing neurons, resulting in denervation supersensitivity.





## ANNUAL REPORT

October 1, 1988 through September 30, 1989

Medical Neurology Branch  
Clinical Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

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ANNUAL REPORT  
October 1, 1988 through September 30, 1989

Medical Neurology Branch  
Clinical Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

Chief, Mark Hallett, M.D.

The Medical Neurology Branch conducts research on human epilepsy, including new approaches to diagnosis and treatment, investigates basic questions related to normal and abnormal neuronal excitability, performs studies on human motor control, investigates cognitive and emotional processes in man, conducts research on memory, perception, language, and problem solving in neurological patients, and investigates immunological and virological aspects of neuromuscular disorders.

The Branch is divided into four sections in addition to the Office of the Chief. The Office of the Chief is composed of two units. Jordan Grafman, Ph.D., is Head of the Cognitive Neuroscience Unit. On October 1, 1988, Marinos Dalakas, M.D., assumed the position of Head of the Neuromuscular Diseases Unit. The Unit conducts research on the immunological and virological aspects of neuromuscular disorders such as polymyositis, inclusion body myositis, post-polio syndrome, and demyelinating polyneuropathies. Research is performed on muscle enzyme histochemistry and immunocytochemistry of muscle and nerve, looking for alterations in the antigenic properties of muscle and Schwann cells. The role of adhesion molecules in the muscle fiber regeneration is extensively studied using specific immune marking for satellite cells. The neuromuscular manifestations of HIV infection are also investigated with a series of clinical, electrophysiological, immunological, virological, and immunochemical studies. The role of antiretroviral drugs in the management of HIV-related neuromuscular diseases is being assessed.

William H. Theodore, M.D., is Chief of the Clinical Epilepsy Section and Acting Chief of the Neuronal Excitability Section. The Clinical Epilepsy Section includes the Unit on Cerebral Blood Flow and Metabolism headed by Dr. Theodore, and the Unit on Neurophysiology headed by Susumu Sato, M.D. Mark Hallett, M.D., is Chief of the Human Motor Control Section. Paul Fedio, Jr., Ph.D., is Chief of the Clinical Neuropsychology Section. During FY 1989 the Speech and Voice Unit of the Human Motor Control Section, under the direction of Christy Ludlow, Ph.D., was transferred to the National Institute on Deafness and Other Communication Disorders (NIDCD).

#### CLINICAL EPILEPSY SECTION

The Clinical Epilepsy Section is undertaking a series of studies using new techniques in order to improve clinical control in patients with refractory seizure problems, as well as to elucidate the pathophysiology of epilepsy. Emphasis is being placed on positron emission tomography (PET) as a technique to investigate basic mechanisms of cerebral metabolism in epilepsy and to assist in the clinical evaluation of patients with severe seizures. Ultrastructural and biochemical investigations of epileptic tissue removed at surgery will be correlated with metabolic findings. Magnetic resonance imaging (MRI) is being used to help elucidate the anatomical substrates of altered physiologic patterns revealed by PET. Nuclear magnetic resonance (NMR)

spectroscopy may be used to study biochemical parameters of human epileptic tissue in vivo.

Patients with severe uncontrolled seizures are admitted to the Clinical Center according to the following criteria: (1) patients with complex partial seizures, especially those who may be candidates for PET scan evaluation and surgical therapy or for trial of an experimental antiepileptic drug; and (2) patients with absence seizures or atonic/myoclonic for studies of cerebral metabolism including the effect of antiepileptic drugs. After seizure frequency and type are characterized by intensive monitoring techniques, the patients are placed in an appropriate research protocol. After the research protocol is completed, each patient's therapeutic regimen is adjusted to obtain optimal seizure control. PET is a technique using the intravenous injection of a radioactive isotope to determine regional rates of cerebral metabolism. The Clinical Epilepsy Section has been using ( $^{18}\text{F}$ )-fluorodeoxyglucose (FDG) to measure the regional cerebral use of glucose. Ongoing studies include (1) patterns of cerebral metabolism in patients with partial, generalized, and atonic/myoclonic seizures; (2) the effect of antiepileptic drugs on cerebral metabolism; (3) effect of chemical seizure activation on cerebral blood flow; (4) correlation of neuropsychological tests with PET results; (5) activation of normal tissue by language tasks; (6) use of  $^{15}\text{O}$  water to study cerebral blood flow; and (7) use of  $^{18}\text{F}$  cycloflor to study opiate receptors in patients with epilepsy.

The role of MRI scanning in seizure disorders is also being investigated. MRI shows more detailed anatomic images than computed tomography (CT) and may detect subtle changes in cerebral density resulting from small gliotic regions which may be epileptogenic. NMR spectroscopy may enable us to detect changes in energy metabolism in vivo in epileptic foci.

A project has been initiated to use sphenoidal, and in some cases subdural, electrodes in the evaluation of potential surgical candidates, coupled with long-term video-electroencephalographic (EEG) recording techniques. These techniques allow the acquisition of EEG data not available via surface recordings. This data is correlated with PET and MRI to obtain the best possible presurgical localization of epileptic foci. A prospective evaluation of the relative value of invasive (subdural) and noninvasive methods of presurgical evaluation is in progress.

During surgery, direct electrocorticographic recordings are made before and after tissue is resected, in order to guide the surgical approach. Tissue from both epileptogenic and nonepileptogenic regions is obtained for biochemical study.

Magnetoencephalography (MEG) is a new approach to the problem of localizing abnormal cerebral potentials which may represent an epileptic focus. Initial studies suggest that MEG may provide more precise three-dimensional information than EEG, allowing detection and localization of epileptic foci in the depths of the brain, without the need for invasive procedures.

Pharmacologic studies in epilepsy continue to concentrate on studies of drug interactions and of new antiepileptic drugs. Patients with uncontrolled seizures, especially complex partial seizures or absence seizures, are accepted for study. Such patients usually have a detailed seizure calendar available prior to entering the study; they enter a week-long period of baseline determination of seizure frequency and blood levels of antiepileptic drugs while in the hospital. Each pharmacologic protocol varies, but all require modification of the antiepileptic regimen and addition of the medication under study. This may be done in single dose or chronic

administration studies depending upon the particular protocol in question. Plasma levels are often drawn daily, and on occasion, much more frequently for specific studies. Following the completion of the pharmacologic protocol, the patient is placed on a regimen which is best suited for the seizure type which has been identified by video tape/telemetered EEG analysis. This regimen is stabilized prior to discharge of the patient.

The Clinical Epilepsy Section has completed a trial of a promising antiepileptic drug developed by Wallace Laboratories. The drug, known as felbamate, is 2-phenyl-1,3-propanediol dicarbamate. In pre-clinical testing the drug is effective in animal models which correlate with partial seizures in man and is quite nontoxic in animals. Evaluation of the protective indices indicate that felbamate has a significant and adequate margin of safety. A randomized placebo-controlled double-blind study was performed. The purpose of this study was to obtain definitive information regarding the efficacy and safety of felbamate in patients with uncontrolled partial seizures who are receiving concomitant carbamazepine therapy. The study uses a unique three period cross-over design in order to eliminate carry-over effects from drug to placebo periods. Data analysis is in progress.

Several new studies of autonomic function in patients with partial seizures have been initiated. Cardiac arrhythmias and neurogenic pulmonary edema may be significant risks in this patient group.

## HUMAN MOTOR CONTROL SECTION

In this year the Speech Pathology Unit moved out of the Section to the new National Institute for Deafness and Other Communication Disorders (NIDCD). A number of collaborative projects are continuing. The Section now consists entirely of what was previously called the Motor Disorders Unit.

### (1) Balance.

The focus of studies in human balance has been the maintenance of upright posture. Quantification of postural sway has been widely applied as a measure of postural stability or balance. Most analyses of sway have been based on an inverted pendulum model, assuming that primary control occurs at the ankle joint. Although this does appear to be a strategy adopted in some situations, our studies show that acceleration of individual body segments characterizes normal upright stance; older normal subjects maintain a more rigid posture, utilizing large and inconsistent adjustments to maintain stability.

Muscular activity during quiet stance has been evaluated for individuals relative to percent of maximum voluntary muscle activity (% MVC). Background postural electromyography (EMG) was consistently on the order of 2% MVC. However, EMG bursts accompanying postural adjustments were as high as 30-40% MVC. The modification of postural control observed in older subjects may represent the individuals' adaptation to changes in the status of the neuromuscular system. High levels of muscular activity may represent too great a challenge to the elderly, resulting in an inability to regain balance when it is suddenly or severely threatened. Additionally, we have evaluated EMG phase relationships among lower leg muscles. While tibialis anterior is rather quiet (1-5% MVC), soleus exhibits low level tonic activity (10-20% MVC) and gastrocnemius is characterized by bursting (10-20% MVC) and quiet periods (1-6% MVC). Gastrocnemius appears to act as a brake to forward



sway, while low level activity in soleus and tibialis provide support at the ankle joint. Ongoing control does not appear to be dominated by these ankle joint muscles as has been previously postulated based on support surface perturbation paradigms.

We have been extending our previous work in the elevation of postural deficits in patients with Parkinson's disease (PD). Measures of standing posture have been demonstrated to be sensitive to clinical change in PD patient status. Presently, we are pursuing a series of single subject studies to evaluate the ability of these measures to indicate drug efficacy. Sensitive three dimensional measures of tremor have been added to this study and we plan to evaluate the effects of a mechanically delivered "pull," reproducing the bedside "push test," in this context. Our previous studies have demonstrated that normal subjects make postural adjustments in advance of voluntary arm movements. These anticipatory postural adjustments (APA) are being studied in patients with PD. Such studies may help us to determine the role of the basal ganglia in the planning and correction of voluntary movements. Patients with cerebellar degeneration will be studied next in this series.

In cooperation with NIAID, we have initiated a pilot study to evaluate the ability of postural measures to detect the emergence of vestibular toxicity in patients with chronic granulomatosis who are treated 4-6 week periods with high doses of gentomycin.

## (2) PET Studies.

Using 18F-2-deoxyglucose for PET scans, we have investigated the regional cerebral metabolism of glucose with voluntary movement and with sensory activation. We have failed to detect any increase in regional cerebral cortical metabolism with simple hand movements or with sensory activation by a vibration device. However, we have seen increase in the metabolism of glucose of both cerebellar hemispheres and the dentate nuclei with the above unilateral voluntary motor activations.

Using H<sub>2</sub>O<sup>15</sup> as a marker for cerebral blood flow, we have looked for changes in the motor and sensory cortex with simple hand movements and with sensory stimulation. We have found an increase in the cerebral blood flow contralateral to voluntary wrist flexion/extension in the motor and sensory cortices. Also, there was increased blood flow of the supplementary motor cortex.

## (3) Voluntary Limb Movements.

Currently, there is a controversy of which cognitive processes occurring in relation to voluntary movement are impaired in patients with PD. While it is clear that these patients have severe motor impairments, the extent to which the motor abnormalities of parkinsonism are associated with deficits in information processing that precede and accompany movement remains unclear. In the previous year, we described a study in which psychophysical measures of reaction time (RT) and movement time were related to circulating levels of l-DOPA. We measured RT when PD patients were required to move either to the right or to the left to a precued target (simple RT task) or to move to a target whose direction became evident only when the signal to move occurred (choice RT task). The distinction between the two tasks was that in the simple RT task, the patients could prepare for the direction of upcoming movement during the delay period, whereas for the choice RT task, the patients had to wait until the end of the delay period to determine the direction of movement. It was found that directional choice RT varied as a function of clinical state, but that simple RT was not affected much by circulating levels of dopamine.

These data point to a role of dopaminergic systems in the basal ganglia as important determinants of directional information processing occurring before movement, since RT, in a complex movement situation, is related to the central availability of dopamine in the normally dopamine depleted parkinsonian patient. Additional experiments illustrated that the concurrent specification of movement amplitude and movement initiation was insensitive to dopamine replacement therapy. This was evident even though the RT of PD patients in the extent choice situation was slower than normal at all medication levels. Another issue concerning patients with PD is whether mechanisms of learning and memory in relation to voluntary movement are impaired. We evaluated the performance of PD patients, and normal controls were asked to make a series of sequential movements with either normal vision or with mirror reversed vision. The time to complete the movement and the accuracy were measured as a function of movement repetition. The patients with PD were able to alter their movement strategy and to learn to perform the movements with mirror reversed vision. This indicates that the basal ganglia are probably not involved in adaptive phenomena related to motor control.

In another series of experiments, the motor functions of patients with cerebellar dysfunction were studied. We evaluated two groups of patients; those with cerebellar hemispheric atrophy and those with olivo-ponto-cerebellar atrophy (OPCA). In these patients, we studied the ability to perform voluntary movement, the size of short and long latency response to muscle stretch, and movement adaptation processes. We studied reflex responses in the wrist flexor and extensor muscles of patients to determine whether short and long latency muscle responses to stretch are altered in cerebellar disease. Responses were elicited when subjects maintained different levels of activation of the extensor and flexor muscles. Subjects were required to resist, assist, or not react to the stretching stimulus. Short latency muscle responses to stretch were suppressed in both groups of cerebellar patients in comparison with normal controls. The long latency response was enhanced in patients with isolated cerebellar disease, but was suppressed in those with OPCA. The results show that the cerebellum influenced short and long latency reflexes.

There exist long-standing questions about the precise role of the cerebellum in coordination. Work by Pellionisz has emphasized the action of the cerebellum in calculating the appropriate distribution of neural activity to various muscle groups involved in a movement. More recently, work by Keele and others, has focused on the possibility that the cerebellum's fundamental computation is that of clocking neural motor control signals. To evaluate the validity of these and other viewpoints, the detailed kinematics of upper extremity movements in two dimensions in human cerebellar patients and in normal controls are being examined. In addition, EMG signals are obtained from involved muscle groups. These data are then used in conjunction with a biomechanical model of the upper extremity to infer the joint torques which are developed during the movement. A comparison is then made between the force patterns in normals and cerebellar patients with regard to magnitude, distribution, and timing. This is essentially an extension to two-joint movements of the work on EMG patterns in single-joint movements performed by Hallett, et. al. The goal is to identify, if possible, the fundamental motor control derangement in cerebellar patients which leads to ataxia in their movements.

#### (4) Evoked Potential Studies.

The optimal interelectrode spacing for topographic brain mapping of human somatosensory cortex was calculated by using the Nyquist distance for the potential of interest. We have determined the Nyquist distances for interelectrode spacing



necessary to record accurately the geographically small components of somatosensory evoked potentials (SEP) in the sensory strip. These distances are less than 3 cm, which is significantly smaller than the 7 cm distance of the 10-20 system. Recordings of SEP in the region made with larger spacings may have significant errors. Recordings made at the Nyquist distance may be precisely interpolated to arbitrary accuracy.

By using this spatial sampling, we could differentiate, in normal volunteers, maps of SEP obtained to stimulation of different fingers, median and ulnar nerve at the wrist or at the elbow, and cutaneous stimulation of the skin overlying forearm, arm, and shoulder. It was also possible to differentiate sensory maps obtained to stimulation of distal and proximal areas of the leg. These topographic maps of SEP were correlated with motor maps of each body part representation as defined by using noninvasive electric and magnetic stimulation.

Four epileptic patients who were going to be operated on, with a grid of subdural electrodes positioned over sensorimotor areas, were studied before and after implantation. Before implantation, SEP from scalp electrodes were recorded to finger, nerves and skin stimulation of the arm. After implantation, SEP were recorded to the same modalities of stimulation from the subdural electrodes. Comparison of both noninvasive and invasive procedures are under way. Mild electric stimuli were delivered through pairs of subdural electrodes precisely localized by X-ray and MRI scans and the signal recorded from scalp electrodes with the purpose of determining the precision of noninvasive techniques for dipole localization. These SEP studies also gave baseline information on the precise localization of sensory representation areas useful for studies on focality of magnetic stimulation and attenuation of somatosensory perception by magnetic stimulation.

In a study under course, four patients with generalized dystonia have been tested and the size of the N30 component of SEP to median nerve stimulation seems to be larger than normal. Further studies are being performed in patients with unilateral dystonia (hand cramps) to determine if the same finding is observed in the dystonic side. Several patients with stroke and amputations have been studied in a project under course. Mapping of SEP has been normal in the two patients with congenital mirror movements suggesting no disturbance in cortical somatotopic organization.

#### (5) Movement Related Potentials.

We have used the standard, widely spaced electrode montage in topographic electroencephalography (EEG) mapping to study the scalp distribution of the terminal phase of the Bereitschaftspotential using a collection window of 499 ms. After establishing normative data on the subpotentials preceding the voluntary index finger movements in normal population, we have been able to do detailed analysis on the generators of the different subpotentials using a tightly spaced electrode montage over the sensorimotor area of the scalp.

We have found differences in the motor cortex activation between dominant and nondominant hand movement execution. Also, we have been able to differentiate motor and sensory deflections from the very controversial motor potential. Most of the work has been done with index finger movements, but also analysis of premovement potentials of foot movements has been done. The development of the method has been sufficient to allow us to study certain patient groups. Small groups of patients with cerebellar atrophy and another with PD have been

evaluated. Typical features of abnormal premovement potentials in PD appear to be short duration, high amplitude potentials with bilateral motor cortex activation when intending to move the more affected side. Cerebellar atrophy appears to lead to decreased amplitude and, especially, to a very diffuse motor cortex activation compared to normal focal activation of the contralateral hand motor area.

#### (6) Stroke.

Preliminary studies of hemiplegic stroke in normals have been initiated and a few patients have been evaluated. Patients have been through a complete clinical evaluation. We have collected data on attempted rapid wrist flexion movements and stretch reflexes of wrist flexor muscles. These patients are also studied with evoked potentials, movement related potentials, and PET studies.

#### (7) Dystonia.

Considerable attention has been paid to the task specific focal dystonias of the hands such as writer's cramp and pianist's cramp. A number of physiological characteristics have been defined. Five elements identified by physiological investigation are indicative of impaired motor control: co-contraction of antagonist muscles, prolongation of EMG bursts, tremor, lack of selectivity in attempts to perform independent finger movements, and failure of willed activity to occur. Gating of somatosensory evoked potentials with voluntary movement is abnormal in that movement of the fifth finger influences the median nerve evoked potential (which it should not do). Abnormalities of the blink reflex have been identified in dystonic disorders. We have verified this in a number of our patients with blepharospasm and spasmodic dysphonia but not in a group of patients with hand cramps. These patients have also been studied with a specific test for analysis of reciprocal inhibition in the arm. Reciprocal inhibition is diminished in the arm which shows the focal dystonia, but not in the arm without dystonia. This finding is similar to the abnormality seen in generalized dystonia. This finding of a physiological abnormality in focal hand cramps is the most objective laboratory abnormality seen in these patients, and is now the strongest evidence against the psychogenic origin on the disorder.

Since it was observed in some cases that a focal dystonia developed after trauma affecting the same region of the body, it seems possible that pain could be a significant factor in its pathogenesis. To test this hypothesis, we have been measuring the reciprocal inhibition of the H-reflex during pain. Reciprocal inhibition was tested as usual and then was repeated after the hand of the subject was immersed in ice and the subject reported a painful feeling. Also, special attention was given to maintain the relaxed state of the arm during the period of pain in the hand. Results at this time are preliminary, and with the exception of the measurements at a delay of 10 ms between the radial and the median nerve stimulation, no clear trend could be seen. At this delay, however, all three subjects showed a tendency toward a less pronounced inhibition with pain than in the control condition.

The electrocutaneous reflex (ECR) consists of a triphasic modulation of ongoing voluntary activity in the hand muscles after stimulation of skin afferents. After stimulation of the index finger and recording from the FDI muscle, there are peaks of excitation at mean latencies of 40 and 66 ms and a peak of inhibition at 51 ms. Similar to the period of inhibition observed in this reflex, an inhibition of the motor evoked potential (MEP) in APB after cortical stimulation has been reported to occur

in normals 17 to 20 ms after stimulation of the index finger. Given a latency of about 20 ms for the MEP in APB after cortical stimulation, this corresponds to a latency of about 40 ms after finger stimulation. In contrast to this inhibition observed in normals, patients with clear signs of parkinsonism showed up to 50% facilitation of the MEP in the same experiment, whereas when they were tested under dopa-therapy, this facilitation was reported to be absent or even reversed. We expect the period of inhibition of the ECR to be altered accordingly in patients with Parkinson's disease. Similar changes might be seen in other movement disorders.

Spasmodic dysphonia, a disorder that has been regarded as being psychogenic for a long time, is now considered to be a focal dystonia that involves mainly the laryngeal muscles. Though the exact pathoanatomic basis is unknown, it is most likely that this disorder, like other focal dystonias, is due to a deficit in the function of the basal ganglia. A common clinical sign in these disorders is the absence of habituation of trigemino-facial reflexes, such as the blink reflex or perioral reflexes, which can be evoked by tapping either the glabella or the skin in the vicinity of the lips. Recent studies on the recovery cycle of the R2 response of the blink reflex in patients with spasmodic dysphonia have demonstrated a loss of inhibitory influence on brainstem interneurons which are conveying the afferent impulse from the trigeminal to the facial nucleus. However, in some patients with spasmodic dysphonia the blink reflex and the blink reflex recovery cycle may be normal, whereas a tapping to the perioral skin evokes increased reflex responses in the perioral muscles indicating an inhomogeneous influence of the basal ganglia on different parts of the somatotopical organized facial nerve nucleus. Thus, a precise electrophysiological assessment of trigemino-facial reflexes of lower face muscles may provide further information on the functional integrity of the trigemino-facial pathways in addition to blink reflex studies.

We developed a technique to evoke perioral reflexes by stimulating branches of the trigeminal nerve (infraorbital and mental nerve) either electrically or indirectly via stimulation of cutaneous receptors by delivering well-defined taps to the skin in the vicinity of the lips, mimicking the clinical test. The reflex responses consisting of an ipsilateral early R1 response, a late bilateral R2 response, and occasionally a rare third response are recorded using surface electrodes. This reflex pattern is very similar to the blink reflex. Presently, we are studying patients who are suffering from focal dystonias with involvement of facial muscles, particularly patients suffering from spasmodic dysphonia. According to the data that are available up to now, changes in recovery cycle and recruitment curves of reflexes following stimulation of the mental nerve are most likely.

In one completed study, we looked at 19 patients with hand cramps, including writer's cramp, typist's cramp, piano and guitar player's cramp. EMGs were recorded while patients performed the task triggering the cramps. Ten patients with dystonic cramps had EMGs with generalized muscle spasms with co-contraction of agonist and antagonist muscles. In three patients with simple cramps that involved one to three fingers, specific muscle groups showed co-contracting bursts that lasted longer than normal. The physiological abnormalities support the interpretation that hand cramp is a focal dystonia, characterized by both excessive muscle activity and defective fine motor control.

#### (8) Botulinum Toxin Treatment for Dystonia.

In a completed study, we evaluated the effects of botulinum toxin injections on 19 patients with hand dystonia. The dystonic muscles were identified by clinical



examination and EMG findings of localized bursts of muscle activation with fine wire electrodes during the tasks that precipitated the dystonia. Injections into the most active muscles were given to each patient every two weeks in increasing doses (up to 20 units the first week, up to 40 units the second week, and up to 80 units the third week) until performance improvement was achieved. Subjective improvement of cramping, pain and/or tension was associated with temporary weakness in injected muscles. Benefit was seen in 16 patients, lasted between 1 and 6 months, and was reproducible.

By now, we have studied thirty seven patients with writer's cramp. All have manifested some improvement. Finding the appropriate dose is tricky and extensor cramps seem more easily treated than flexor cramps. The EMG appearance of the spasm is the same both before and after therapy suggesting that the major mode of action is to weaken the muscle and not to reduce the amount of cramping. Botulinum toxin has proven to be both safe and effective.

#### (9) Cortical Stimulation.

We have succeeded in mapping the hand, arm, leg and mouth areas of the human motor cortex in normal volunteers and correlating these motor maps with the sensory maps as defined by using somatosensory evoked potentials. At this time we are studying changes in these sensorimotor maps in patients with stroke, and with different types of amputations. We have found that patients with congenital mirror movements have a bilateral cortical representation of each hand in the motor cortex. Also, that they have physiologically active and fast conducting connections between the motor cortex and ipsilateral muscles in the upper extremity. We have also found that EEGs do not change after a session of cortical stimulation in normal volunteers, which indicates the safety of the procedure. We have established normative data for our own laboratory for measurement of motor conduction velocities using electrical stimulation.

We are now using magnetic stimulation for the purpose of stimulating focally different parts of the brain. We have shown it possible to map the representation of different body parts in the human motor cortex using this technique. We have also determined that these maps are precise. This was accomplished by mapping the hand motor representation areas with noninvasive magnetic stimulation and comparing the results with those obtained when using direct stimulation through subdural electrodes. We have also developed a model capable of predicting the focality of stimulation of different magnetic coils. We have also studied hemispheric dominance for laryngeal muscles finding that there seems to be bilateral projections from both hemispheres to motoneurons controlling muscles in both sides of the larynx and that stimulation of the left hemisphere activates a larger percentage of the motoneuron pool bilaterally.

We studied the effects of magnetic stimulation on somatosensory perception. Somatosensory perception was attenuated when scalp stimulation was delivered close to a peripheral stimulus to the finger. This effect had topographic specificity being produced by scalp stimulation of restricted scalp positions contralateral to the finger stimulated, was maximal with low intensities of finger stimulation and high intensities of magnetic stimulation (usually over motor threshold), and could also be produced in the absence of motor evoked responses in peripheral hand muscles. These results suggest that a focal cortical stimulus can briefly attenuate somatosensory perception before, during, and after cortical arrival of a somatosensory afferent

volley, and that this effect appears to be due, at least in part, to stimulation of sensory representation areas.

We have also used magnetic stimulation to probe the processes in motor cortex during a reaction time task in patients with PD. Such patients have slow reaction time. We found that there is prolongation in the time it takes the motor cortex to go from initial excitability to a level to produce the motor command.

#### (10) Trial of Isoniazid for Action Tremor.

Twenty patients have been studied. The major diagnostic category has been essential tremor, and other categories include post-traumatic tremor and postural tremor associated with parkinsonism. Preliminarily, we have seen some clinical improvement in a few of the patients with essential tremor and in one of the patients with parkinsonism. Quantitative statistical analysis is in progress and a report will be written.

### CLINICAL NEUROPSYCHOLOGY SECTION

Neuroscientific studies conducted by members of the Clinical Neuropsychology Section have explored the reciprocal functional specialization of left and right brain mechanisms that integrate and guide cognitive and emotional behavior. The presence of brain disorders introduces disruptive changes in memory, perception and other cognitive domains, and at the same time, causes serious upheaval in personal and social arenas. These cross-disciplinary studies provide a better understanding into the basic mechanisms of human behavior, and how brain injury expresses in learning disabilities, affective disorders and schizophrenia, and the dysfunctions of aging, notably dementia.

In one protocol, patients with complex partial seizures originating from the left or right temporal lobe were evaluated with neuropsychological tests before and after unilateral temporal lobectomy and during specialized techniques, including brain stimulation, intracarotid amytal injection (Wada), and physiological procedures (EEG, electrodermal responses).

These studies have demonstrated that the mnemonic services of the temporal lobe supporting declarative and incidental memory depend upon the integrity of mesial structures, especially the hippocampus. Following therapeutic unilateral temporal lobectomy, patients did poorly with cross-modal learning which is crucial to linguistic and lexical processes. The post-operative decline seems strongly linked to whether or not the amygdala was removed at surgery, which reinforces the notion that this mesial structure integrates sensory information, often with emotional coloration. Specifically, left temporal patients did poorly with episodic memory tasks and in depositing and retrieving information from memory registers, particularly after lengthy delays; retroactive interference contributed to this problem. These patients were not advantaged by internal cues, such as ability to cluster on the basis of semantic categories. In contrast, right temporal patients did well to define and apply coding rules to facilitate learning and recall.

Experimental and autobiographical memory was found to be highly vulnerable to temporal lobe injury, with evidence of disturbances in the chronology of occurrence, more so with left than with right temporal lobectomy. Using the patient to generate mnemonic cues, preliminary data suggests that conceptual and data



generated ideas from internal or external sources are properly balanced by the left and right temporal lobe, respectively. The primacy of interhemispheric exchange of information, however, favors the left hemisphere as the ultimate recipient of knowledge encoded and processed by either cerebral hemisphere. This may explain the appearance of marked personal- social-cognitive impairment following left brain injury, far in excess of the effects of dysphasia and kindred disorders.

Whereas emphasis in brain research in the Section is biased to explore the cognitive processes of the left hemisphere, owing to the primacy of language, we have also evolved several techniques to examine the dominant contributions of the right brain, not only to intellectual, but to emotional functions. One approach has revealed that the right temporal lobe mediates paralinguistic processes such as prosody and speech perception, preferentially attuned to emotionally charged stimuli. This brain region is also dedicated to spatial knowledge and analysis of visual information dealing with location, judgment and discrimination, and with the appraisal of cognitive and emotional features. The implications of this in reading and kindred lexical processes, and in the perception and interpretation of emotional nuances, is being studied and may be extended to patients with dementia who present selective atrophy in the left or right posterior quadrant of the brain.

In the emotional domain, the right temporal lobe is sensitive to stimuli that may threaten or enhance the well-being of the individual in relation to the external environment. The left temporal lobe is more adept to manage the internal milieu and logic of cognition, giving it a dominant role in "consciousness."

Recently, a series of studies explored neurolinguistic changes accompanying insult to regions outside the classic speech and language zones. Semantic processing was disrupted by electrical stimulation of primary cortical speech-language areas, leading to receptive and expressive impairment for materials delivered via auditory and visual (reading) channels. Stimulation of the inferior-posterior temporal cortex, usually inaccessible to exploration except by flap electrodes, elicited distinct errors in naming, reading and memory. What was unusual was that aphasia and amnesia were dissociable; that is, the patient, while being able to speak, failed to name objects during stimulation but correctly recalled their names after stimulation. This is not a common pattern following stimulation of the more classic brain sites, and confirms memory (retrieval) as one of the main functions of the region and offers a basis for some errors in anomia. Since there is a tendency to extirpate the temporal lobe more conservatively along the superior gyrus, and more aggressively along the inferior gyrus, post-operative dysphasia may be related to resection of this region in the posterior-inferior temporal cortex.

A series of special procedures are being developed to investigate how syntactical and phonological rules are organized and applied by frontal brain (Broca's) region. The paralinguistic contributions of the right hemisphere involve prosody, but the basic mechanisms deal with spatial disembedding and closure, even with verbal material. During stimulation, for example, the patient may fail to interpret the intent of speakers based on voice rate and intonation, while being spared dysphasia. This is highly relevant and better explains how the right brain serves emotional perception and expression.

As part of a preoperative, diagnostic arteriographic study, epileptic patients receive intracarotid amytal (Wada) to evaluate the functional integrity of language and memory by the left and right brain. A bold and innovative evaluative paradigm was developed in the Section to assess how the left and right brain perceive and

remember linguistic materials, utilizing phonological and spatial attributes while naming achromatic, incongruous and congruously colored objects, and while reading words in print, cursive script, and a disembedding format.

Dysphasia and dyslexia accompanied the left injection and patients did equally poorly to name achromatic and both types of chromatic objects. After the dysphasia resolved, cursive and embedded words were read correctly, but there were phonological repetitions and distortions of carrier words. There were few naming errors with the right injection, but patients did poorly to name objects in idiosyncratic color, being unimpaired to name normally colored and achromatic pictorial objects. Patients after right injection usually did very well to read printed and cursive words, but failed to disembed by reading only the carrier and not the embedded words. The results clearly showed that the two hemispheres work in concert, and that phonological and semantic rules observed by the left brain may be supplemented by spatial dominant rules imposed by right brain, so that early lesions to either hemisphere may create different forms of speaking, reading, or writing disorders.

In another main research thrust, neuropsychological studies have been extended to examine changes accompanying the normal aging process and include comprehensive investigations of dementia. One set of studies was developed to examine the character of defects of patients with unilateral or hemi-parkinsonian disorders. Preliminary analyses have revealed the emergence of defects in attention and integration, and showed that cognitive processing becomes less flexible in decision making and rendering judgments. There was, however, no classic functional asymmetry like that seen with patients presenting unilateral cortical lesions.

In dementia of Alzheimer's (AD) and Huntington's (HD) diseases, episodic memory defects for various materials were commonplace. To probe the nature of this defect, fundamental questions about the integrity of perceptual processes were formulated and tackled with special noninvasive interhemispheric tests (tachistoscopic recognition and dichotic listening). AD patients manifested appreciable perceptual defects in the absence of sensory losses, and had difficulty interpreting and remembering novel material. This was replicated with dichotic listening where AD patients were unable to selectively allocate attention, particularly from material directed to the left ear (right brain). In contrast, HD patients did poorly with processing and manipulating visuospatial materials, implicating involvement of different cortical and subcortical systems in these major dementia disorders. Attentional dysfunctioning, therefore, contributes much to the defective performance of patients with dementia.

Dopamine replacement therapy with Parkinson disease (PD) patients continues to provide new insight into cognitive and motoric modulation. PD patients, studied during stimulated and unstimulated levodopa states, displayed profound changes in motor activities and proficiency in delayed verbal memory. These differential contributions of nigrostriatal and mesolimbic/cortical systems remain an attractive explanation to account for dissociable motoric and memory gains. The issue of effortful as opposed to automatic memory will be explored, and as well, hypotheses about bipolar mood shifts and organic psychosyndromes of this disorder will be challenged.

The specificity and magnitude of cognitive deficits in PD has been subject to increasing debate. To further address this question, PD patients with exceptional professional and educational distinction who continue to function in leadership

positions, were compared with matched nonaffected individuals. The patients showed relative retention of verbal skills and higher executive function, but exhibited significant decrements in episodic memory as well as visuospatial integration. Although losses on tasks requiring speed and manual manipulation were likely reflective of motoric problems, perception errors were more likely reflective of cognitive losses.

Another investigative question asked whether in early Parkinson's disease with asymmetric symptomatology there were analogs of left-right brain functional differences in the cognitive realm. We examined patients with predominantly left or right lateralized motor involvement and matched controls by means of neuropsychological testing and positron emission tomography (PET) with [<sup>18</sup>F]-fluorodeoxyglucose (FDG). The neuropsychological profile of the two patient groups failed to show any consistent right-left differences, although both demonstrated selective deficits in verbally mediated episodic memory and visuospatial functions compared to normal controls. PET scans revealed systematic unilateral hypermetabolism in the basal ganglia, particularly in the lenticular nuclei contralateral to the predominant motor signs, but no right-left cortical differences. Thus, while PET data confirmed lateralized metabolic hyperfunction in the basal ganglia, cortical metabolic values, as well as neurobehavioral indices, did not detect lateralized dysfunctions.

As noted above, visuospatial deficits are commonly cited in PD. To quantitate differences between PD and AD dementia, patients matched for overall dementia severity, age and education, were contrasted neuropsychologically. Achievements with visuospatial and memory tasks were significantly compromised in both patient groups. Visuospatial tasks demanding executive functions were defective in both patient groups, but tended to be more impaired in PD. PD dementia appears not to share features compared to AD and shows disproportionately greater disruption in "executive function" and less severe decrements in visuospatial tasks with generic memory components. Parkinsonian dementia, however, appears to impart distinct features, suggestive of greater frontal neuropathogenic involvement.

Perception and attention assessed by dichotic listening tasks, were compared in AD and normal subjects, manipulating list length and order of recall. Alzheimer patients tended to show qualitatively similar, but significantly worse performance with increasing list length as well as stimulus content (semantic vs. phonemic vs. unrelated items). Furthermore, these patients were unable to selectively attend to one or another auditory channel and could not increase a right or left ear advantage, a task easily mastered by normal subjects. As such, AD disrupts brain mechanisms involved in the selective allocation of attention, which may provide a basis for the pervasive amnesic defects associated with the disease.

The character of visuospatial dysfunction in HD was evaluated in relation to actuarial indices, such as symptom duration, age at onset, and severity. Factor analytic procedures indicated that perceptuomotor capacity (Factor 1) as well as the ability to manipulate spatial information (Factor 3) were markedly affected. In contrast, spatial discrimination (Factor 2) appeared to remain relatively intact. Age at onset had no relationship with these variables, while severity of dementia was significantly related with overall impairment of visuospatial processing. Most importantly, duration of symptoms was associated with the declining ability of patients to mentally manipulate spatial memoranda. The fact that circumscribed visuospatial impairment was present in HD patients, may have important consequences for the evaluation of efficacy of experimental therapeutic interventions.



Response fluctuations in motor function and complicating long-term dopaminomimetic therapy of PD, may extend to the cognitive realm. To evaluate levodopa treatment effects on attention as well as acquisition and retrieval (memory), PD patients were examined while medicated with levodopa/carbidopa ("on") and when the medication's antiparkinsonian effect had worn off ("off"). Significant cognitive differences emerged only on the delayed recall of complex verbal materials where patients performed better during the "on" than the "off" state. Comparison of change scores across states (administration or withholding of levodopa/carbidopa between acquisition and retrieval, "off" to "on" or "on" to "off") revealed no substantial differences as a function of dopaminomimetic therapy. Thus, slight changes in cognition are associated with dopaminomimetic therapy of Parkinson's disease, but it may be task-specific.

The functional role of the noradrenergic system in learning and memory in the experimental animal, together with reports of relative sparing of cortical noradrenergic receptors in AD, encouraged double-blind, placebo controlled clinical trials of two noradrenomimetic agents, clonidine and guanfacine. As cortical noradrenergic receptors appear relatively spared in AD, we attempted a double blind therapeutic trial with clonidine, a centrally active alpha-2 receptor agonist. Verbal and visuospatial memory as well as attention were assessed, and a small improvement was seen in verbal memory with low dose treatment; clinically observable effects were limited to decreased blood pressure and mild sedation at higher doses. These disappointing results might reflect dose limiting side effects as well as the need for alternative approaches to noradrenomimetic therapy; clonidine appears to confer modest therapeutic benefits.

Developmental neuropsychological anomalies provide an opportunity to study altered cerebral organization and compensation of functions following early brain injury. A new project has been initiated to assess patients with metabolic disorders (Gaucher's, Niemann-Pick). Whereas, the deteriorative decline and emergence of dementia is predicted for Gaucher's Type III disease, the character and manner of progressive unfolding deficits and compensation remain unknown. Apart from subtle oculomotor changes, Gaucher's Type I disease patients reportedly are asymptomatic in neuropsychological terms. However, mild to moderate learning disabilities by these patients have been identified in the areas of perceptuomotor integration; this translates into spelling and writing disorders while basic language processes remain relatively intact.

The impact of hormonal agents upon neuropsychological processes was tackled in a study of patients with Turner's syndrome. Preliminary analysis indicated that these patients exhibit patterns and responsiveness resembling those of younger children. In an independent neuropsychological study nearing completion, attentional losses and visuospatial dysfunctioning dominated the test profile.

The role of frontal-striatal structures in neuropsychiatric disorders continues to gather empirical support. This structurofunctional hypothesis was examined in a group of children with an obsessive-compulsive disorder. These patients presented enlarged ventricular indices and selective neuropsychological defects, primarily with tasks demanding frontal participation. The obsessive-compulsive children had difficulty in generating and sustaining new ideas and concepts, and were unable to overcome rigidity in set and approach. Further analysis of symptom subtypes failed to establish any link between obsession or compulsion and brain asymmetry.

## NEURONAL EXCITABILITY SECTION

During the current fiscal year, the Neuronal Excitability Section, under the direction of Dr. Suzan Nadi, conducted research in the following areas of the chemical basis of epilepsy:

(1) The alterations in the levels of neurotransmitters, neuromodulators, their receptors in the course of the development of kindling in the rat.

Preliminary data shows, for example, that the changes in the levels of somatostatin start to become detectable in stage 3 and become more marked as the rat progresses to stage 5. Future studies in this direction will help correlate how the seizure development affects the transmitters.

(2) The interactions of different transmitters at the receptor level (via second messengers) in the human and kindled rat brain.

Such studies carried out on in vitro slices will help us better understand how this interaction is altered to make certain areas of the brain more excitable. Preliminary data suggests that NMDA receptors respond with an increased second messenger synthesis in the presence of norepinephrine. This effect is more accentuated in the kindled rat brain than the sham operated brain. Similar studies are under way for the human brain slices.

(3) Studies of the expression of the mRNA for specific peptides such as c-fos, somatostatin, enkephalin, and glutamate decarboxylase are underway in kindled rats.

Preliminary data shows an increase in enkephalin in the dentate gyrus region of the hippocampus, and an increase in somatostatin mRNA in the cortex and substantia nigra of the rat. These results agree well with previous radioimmunoassay results. Glutamate decarboxylase mRNA did not change in the brain region studied. These findings also correlate well with enzyme studies. Future studies are underway where the regulatory processes governing the expression of these genes will be studied. In addition, hybridization studies in both kindled rat and human brain will help identify the cellular localization of the mRNA alterations.

(4) Autoradiography of receptors in the human brain.

These studies using in vitro receptor binding will help elucidate the distribution of the receptor and the localization in the temporal neocortex of the altered binding sites. There are no differences in the spiking versus non spiking cortex. This data would be in agreement with the increased opiate mRNA activity observed in earlier studies. In addition, NMDA receptors will be studied using the same methodology.

During the past year, Dr. Michael Rogawski continued pharmacological studies of voltage-dependent  $K^+$  channels and N-methyl-D-aspartate (NMDA) gated cation channels in cultured mammalian CNS neurons using whole cell voltage-clamp and single channel recording techniques. The aim of these studies was to explore new strategies for the rational development of antiepileptic drugs based upon their interaction with ion channel systems that are critical to the regulation of CNS neuronal excitability and epileptogenesis. Work was focused in three areas: (1)  $K^+$  channel activator drugs, (2) phencyclidine (PCP) related drugs, and (3) antagonists of the glycine modulatory site on the NMDA receptor. In addition, studies were



completed on voltage-dependent  $\text{Ca}^{2+}$  channels in enzymatically isolated thalamic neurons. Finally, Drs. Rogawski and Yamaguchi, in conjunction with the Laboratory of Medicinal Chemistry, NIDDK, continued the evaluation of a series of novel phenicyclidine analogs for their activity as anticonvulsant agents.

#### (1) $\text{K}^+$ Channel Activator Drugs

Voltage-dependent  $\text{K}^+$  channels regulate neuronal excitability by acting to repolarize the neuronal membrane. Recently, several different antihypertensive drugs have been shown to stimulate the opening of  $\text{K}^+$  channels in muscle cells. Drs. Politi and Rogawski observed that one of these drugs, cromakalim, promotes the opening of tetraethylammonium-sensitive voltage-dependent  $\text{K}^+$  channels in cultured hippocampal neurons. Studies are underway to determine the specific  $\text{K}^+$  channel type affected by cromakalim and the mechanism of action of the drug. In addition, the potential anticonvulsant activity of cromakalim and related compound is being evaluated.

#### (2) Cellular Electropharmacology of PCP and Related Compounds

Excitatory neurotransmission mediated by NMDA receptors plays a critical role in epileptogenesis. Steric blockers of the NMDA receptor-associated ion channel, such as the dissociative anesthetic PCP, are powerful anticonvulsants. However, side effects, including ataxia and cognitive disturbances, limit the practical usefulness of these drugs in the treatment of seizure disorders. During the reporting period, Drs. French-Mullen and Rogawski completed a comparative study of the interaction of PCP with voltage-dependent  $\text{K}^+$  channels and NMDA receptor-channels in cultured hippocampal neurons. The main conclusions of this study were (a) the affinity of PCP for NMDA channels is 13 times greater than for  $\text{K}^+$  channels, (b) the drug blocks the two channel types by distinct biophysical mechanisms, and (c) the pharmacological properties of the PCP acceptor site on the two channel types is different. These observations further support the concept that the main pharmacological actions of PCP, including its anticonvulsant and ataxia-inducing activity, occur as a result of its interaction with NMDA receptors.

#### (3) Synthesis and Evaluation of PCP and MK-801 Analogs

During the past year, the collaborative program with the Laboratory of Medicinal Chemistry, NIDDK, for the synthesis and evaluation of novel PCP derivatives was continued. The compounds were screened in several animal seizure models and in a motor toxicity test. Several compounds were identified that showed a three- to four-fold improvement in therapeutic index for seizure protection compared with PCP. In addition, studies were begun on a series of compounds related to the potent NMDA-receptor channel blocker MK-801. Of particular interest is 5-aminocarbonyl-5H-dibenzo[a,d]cyclohepten-5,10-imine which showed a six-fold improvement in therapeutic index compared with PCP. A patent on this compound has been filed.

#### (4) Characterization of Membrane Currents in Thalamic Neurons

Drs. Suzuki and Rogawski completed studies on the ionic currents in isolated guinea pig thalamic neurons. The low threshold spike (LTS) in these cells was found to be mediated primarily by T-type (low voltage activated, transient, dihydropyridine-insensitive)  $\text{Ca}^{2+}$  channels. The LTS is responsible for the transition between tonic and burst firing, and may play a critical role in the generation of absence (petit mal)

seizures. T-type  $\text{Ca}^{2+}$  channels may be an important target for anti-absence drugs (such as ethosuximide and dimethadione). Studies have also been completed on the characterization of voltage-dependent  $\text{K}^{+}$  channels in isolated thalamic neurons. Using this information, a mathematical model of the LTS was developed in collaboration with Drs. Michael Weinstein and John Rinzel, Mathematical Research Branch, NIDDK.

## COGNITIVE NEUROSCIENCE UNIT, OFFICE OF THE CHIEF

The primary objectives of the Cognitive Neuroscience Unit are to identify and model the components of information processing, the cognitive computations that underlie each component, and the categories and architecture of knowledge representation systems. Furthermore, we make an effort to map cognitive processes onto human brain physiology, structures, and systems.

Investigators in the Cognitive Neuroscience Unit are currently studying a wide range of cognitive processes including problem solving and reasoning; memory and knowledge representation; number processing and calculation; reading, writing, and naming; visual perception; and the relationship of mood state and emotions to stored knowledge. Although many of these studies utilize young and old normal subjects, the majority of our studies are conducted with central nervous system (CNS) impaired patients. CNS impaired patients are studied because their cognitive deficit pattern often implies dissociations between types of information processing components, cognitive computational properties, or knowledge domains. Thus, not only can such patients teach us about the structure of cognition on the basis of their dissociations (and associations too), but the nature and direction of the dissociations may lead to inferences regarding the contribution of anatomical and neurotransmitter systems to cognitive processing. Both single-case within-subject and group study designs are utilized.

The usefulness of studying the components of information processing is supported by several lines of research. For example, the study of memory in Multiple Sclerosis patients has narrowed their memory processing deficits to two components: the articulatory rehearsal loop in working memory which temporarily stores information in a buffer when it cannot be processed on-line, and a post-representation retrieval pathway. Dr. Ray Johnson, Jr. has demonstrated that event-related potentials provide a physiological index of the temporal course of information processing with specific wave-forms and their topography associated with particular information processing components. Information processing components such as the articulatory loop in working memory must have computational properties that transforms and decodes information so that it may be stored in a particular representation. Error analysis in CNS impaired patients has provided clues as to the nature of the computational processes. For example, Dr. Rhonda Friedman is currently analyzing different forms of acquired reading, writing, and naming disorders using error analyses to identify both the disordered component (e.g., semantic lexicon) as well as the probable impaired computational property (e.g., activation of associational links between stored lexical items). Dr. Francois Lalonde is initiating a study to observe the effects of damage to an early visual information processing component (i.e., visual-spatial contrast sensitivity analysis) on later visual information processing components (i.e., form and object discrimination and recognition). If patients have a problem in visual-spatial contrast sensitivity, Lalonde will attempt to identify which computational property (i.e., specific contrast sensitivity channel) is responsible. Finally, Dr. Rhonda Friedman, using priming tasks,

and Dr. Jordan Grafman, using tasks requiring retrieval of items from different domains of knowledge, are mapping out the structure and categories of knowledge representation.

As a result of these studies, we have been able to tentatively assign cognitive components and knowledge representation systems to brain locations. For example, structural analysis of stimuli takes place in the posterior cortex in regions distinct from where meaningful analysis of stimuli takes place. In addition, the more complex the nature of stimulus representation (e.g., schemas), the more anterior in the brain is its representation. Representational systems are organized both serially and in parallel, can be activated in parallel, are partially information redundant, and while informationally hierarchical, can be activated selectively via attentional mechanisms. Basal ganglia structures appear to aid in the execution of representations (e.g., via motor procedures or cognitive planning). The involvement of many different neural structures and neurotransmitter systems in cognition are currently being studied. While we are concerned with specifying the cognitive components of specific neural systems and structures, we also expect in the next few years to describe broader principles of cognitive processing and representational knowledge and to map these broad principles to brain chemo- and neuroanatomy. As alluded to above, specific projects carried out in the Cognitive Neuroscience Unit have supported the conception of cognitive components, computational properties, and knowledge domains that are at the heart of the models of cognition developed by Unit members.

#### NEUROMUSCULAR DISEASES UNIT, OFFICE OF THE CHIEF

The Neuromuscular Diseases Unit conducts clinical studies and laboratory investigations to determine etiology (infection/immunity and/or genetics) of patients with neuromuscular disorders and explore new therapeutic modalities. Current studies include (1) motor neuron disease syndromes such as amyotrophic lateral sclerosis (ALS) and post polio syndrome; (2) demyelinating polyneuropathies; (3) neuromuscular diseases associated with HIV infection; (4) Duchenne's muscular dystrophy; (4) experimental models of retroviruses-induced polymyositis; (5) studies on muscle regeneration; and (6) studies involving the interactions of the lymphoid system with the central or peripheral nervous system.

We have defined the clinical symptomatology of the post-polio syndrome and provided evidence that the site of pathologic involvement is in the distal nerve terminals. We have found changes in all the muscles regardless of the presence of new symptoms suggestive of an ongoing neuronal dysfunction which appears to continue slowly since the original polio attack. Abnormal immunoregulatory mechanisms may also play a role in the manifestation of the symptoms based on the presence of inflammation in the histological specimens of muscle biopsies and spinal cords and abnormal lymphocyte subsets in the circulation.

We have studied the metabolic activity of the cortex in ALS patients and correlated the glucose utilization of the cortical neurons with the histopathological findings of the same brains at autopsy. We found that ALS is a complex disorder affecting multiple cortical regions that extend beyond the resolution of routine histopathology.

In an effort to determine if in Duchenne's muscular dystrophy the weakness is due to absent dystrophin or to loss of muscle fibers, we measured the force generated by



skinned muscle fibers and correlated it with the composition of muscle proteins in the same fibers separated electrophoretically. We found that in Duchenne's dystrophy the remaining muscle fibers generate normal force in spite of the absence of dystrophin.

We have defined the spectrum of neuromuscular diseases associated with HIV infection and investigated the immunopathogenesis of these disorders in an effort to find effective therapies. We found that two antiretroviral drugs, AZT and DDC, currently used in the treatment of AIDS, can cause either a painful destructive mitochondrial myopathy (AZT) or a painful axonal neuropathy (DDC).

Using antibodies to thymosin hormones, thymosin alpha 1 and beta 4, we have demonstrated that the myelin producing cells in the CNS (oligodendrocytes) and PNS (Schwann cells) share common antigenic determinants with cells of the lymphoid system. These observations are helpful to understand the immune mechanisms of demyelination.

The IgM paraprotein associated with demyelinating polyneuropathy was found to be an antibody against peripheral nerve glycolipids. When injected intraneurally in the sciatic nerve of the cat, the IgM induced demyelination suggesting that this protein is responsible for the cause of the demyelinating neuropathy.

In an effort to define the type of amyloid protein that causes amyloid deposition in patients with amyloid polyneuropathy, we have examined immunocytochemically the deposited amyloid for the presence of several amyloidogenic proteins. The protein transthyretin was found to be the major component in the hereditary form of amyloid neuropathy. Specific point mutations were identified in the extracted transthyretin from one family member. This information will help us to perform genetic studies to identify asymptomatic family members.

The cellular events of muscle fiber regeneration are being examined using monoclonal antibodies that recognize satellite muscle cells. Adhesion molecules N-CAM, I-CAM, Leu-19 and other common antigens shared between regenerating muscle fibers, satellite cells and lymphoid cells are examined in the muscle biopsies of patients with various neuromuscular diseases and their experimental models.

We have been exploring a series of new therapies in patients with polymyositis or demyelinating polyneuropathy refractory to available immunotherapies. Studies are conducted with combination of intravenous methotrexate, plasmaphereses and azathioprine. The role of intravenous immune gamma globulin is also studied. Our preliminary findings indicate that gamma globulin is effective in the treatment of patients with paraproteinemic polyneuropathies.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02318-12 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antiepileptic Drugs

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William H. Theodore, M.D. Chief, CES MNB NINDS

Others: Susumu Sato, M.D. Chief, EEG LAB OCD NINDS  
Paul Fedio, Ph.D. Chief, CNS MNB NINDS

## COOPERATING UNITS (if any)

Office of The Clinical Director, NINDS

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Clinical Epilepsy Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

1.2

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒

(a) Human subjects

☐

(b) Human tissues

☐

(c) Neither

☒

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current studies include a double-blind randomized placebo controlled trial of felbamate. The unique three period cross-over design allows unbiased estimates of drug effects even in the presence of a carry-over effect from one period to the next.

The first study has been completed, and data analysis is in progress. We have evaluated the effect of valproic acid on cerebral glucose metabolism, using positron emission tomography (PET). This drug is of particular interest due to its possible inhibition of GABA degradative enzymes. We are comparing its effect to that of phenytoin, carbamazepine, and phenobarbital. We are evaluating optimal therapy for the Lennox-Gastaut Syndrome, comparing carbamazepine plus valproic acid to valproic acid alone.



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|---|-----------------------------------|--|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                                   | <b>PROJECT NUMBER</b><br><br>Z01 NS 02236-14 MNB |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989   |                                   |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Diagnostic and Therapeutic Reevaluation of Patients With Intractable Epilepsy   |                                   |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |                                   |  |
| PI:   | William Theodore, M.D. Chief, CES | MNB NINDS  |
| Others:   | Susumu Sato, M.D. Chief, EEG Lab  | OCD NINDS  |
|   | Paul Fedio, Ph.D. Chief, CNS      | MNB NINDS  |
| <b>COOPERATING UNITS</b> (if any)<br>Office of The Clinical Director, NINDS   |                                   |  |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR   |                                   |  |
| <b>SECTION</b><br>Clinical Epilepsy Section   |                                   |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892   |                                   |  |
| TOTAL MAN-YEARS:  | 1.0                               | PROFESSIONAL: 1.0<br>OTHER: 0                    |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither<br><input checked="" type="checkbox"/> (a1) Minors<br><input type="checkbox"/> (a2) Interviews  |                                   |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br>The Clinical Epilepsy Section has been developing and testing new techniques to improve seizure control, medication tolerance, and rehabilitation in patients with severe <u>epilepsy</u> . Patients with <u>uncontrolled seizures</u> are admitted for a complete evaluation, including simultaneous <u>video and telemetered electroencephalographic (EEG) recording</u> of seizures, daily determinations of <u>antiepileptic drug serum concentrations</u> , <u>positron emission tomography (PET)</u> , <u>magnetic resonance imaging (MRI)</u> , and <u>magnetoencephalography (MEG)</u> . A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy. <u>Cerebrospinal fluid (CSF) biochemistry</u> , and <u>neurochemistry of temporal lobe specimens resected</u> from patients with uncontrolled seizures are being studied. PET in patients with localized brain lesions has demonstrated focal <u>hypometabolic</u> cerebral areas corresponding to the interictal seizure EEG focus. In some patients, PET has been able to detect a focus when other methods have failed. Studies of patients during partial seizures have shown a change from hypo- to <u>hypermetabolism</u> at the site of the focus. In the <u>Lennox-Gastaut syndrome</u> , PET has revealed the existence of two separate metabolic patterns despite clinical seizure similarity. PET studies allow more definitive identification of the <u>epileptic lesion</u> and suggest new avenues of investigation into the basic mechanisms of the epilepsies. MRI may show small structural lesions underlying PET hypometabolism even when computed tomography (CT) is normal. Further studies will elucidate the relation of <u>metabolic</u> and <u>pathologic</u> changes. MEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the <u>spatial distribution of epileptiform discharges</u> in cortical depths; MEG may be superior. |                                   |  |
| 21-MNB/DIR  |                                   |  |

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

**PROJECT NUMBER**  
 Z01 NS 02667-05 MNB

**PERIOD COVERED**

October 1, 1988 through September 30, 1989

**TITLE OF PROJECT** (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Involuntary Movements

**PRINCIPAL INVESTIGATOR** (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                     |                    |      |      |     |       |
|---------|---------------------|--------------------|------|------|-----|-------|
| P.I.:   | Mark Hallett, M.D.  | Clinical Director  | OCD  | ODIR | DIR | NINDS |
|         |                     | Chief              | HMCS | MNB  | DIR | NINDS |
| Others: | Leo Cohen, M.D.     | Visiting Scientist | HMCS | MNB  | DIR | NINDS |
|         | Jerome Sanes, Ph.D. | Special Expert     | HMCS | MNB  | DIR | NINDS |
|         | Ina Tarkka, Ph.D.   | Special Volunteer  | HMCS | MNB  | DIR | NINDS |
|         | Peter Fuhr, M.D.    | Special Volunteer  | HMCS | MNB  | DIR | NINDS |
|         | *                   |                    |      |      |     |       |

**COOPERATING UNITS** (if any)

Department of Nuclear Medicine, Clinical Center

**LAB/BRANCH**

Medical Neurology Branch, CNP, DIR

**SECTION**

Human Motor Control Section

**INSTITUTE AND LOCATION**

NINDS, NIH, Bethesda, Maryland 20892

**TOTAL MAN-YEARS:**

1.0

**PROFESSIONAL:**

0.9

**OTHER:**

0.1

**CHECK APPROPRIATE BOX(ES)**

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

**SUMMARY OF WORK** (Use standard unreduced type. Do not exceed the space provided.)

Involuntary movements have often been difficult to classify clinically. Clinical and physiological analysis of a continuing series of patients has led to new classifications and pathophysiological insights. Dystonias have been the focus of our recent work.

Task specific focal dystonias of the hands such as writer's cramp and pianist's cramp have been analyzed and a number of physiological characteristics have been defined. There appears to be diminished ability to control the fingers independently and gating of somatosensory evoked potentials with voluntary movement is abnormal. The spasms themselves have been characterized into different patterns. Abnormalities of the blink reflex have been identified in dystonic disorders. We have verified this in a number of our own patients and are now applying this test to the patients with focal hand cramps. These patients have also been studied with a specific test for analysis of reciprocal inhibition in the arm. Reciprocal inhibition is diminished in the arm which shows the focal dystonia, but not in the arm which is not dystonic. This finding of a physiological abnormality in focal hand cramps is the most objective laboratory abnormality seen in these patients, and is now the strongest evidence against the psychogenic origin on the disorder. Since trauma often precedes focal dystonia, we are evaluating the influence of pain on reciprocal inhibition.

We are developing other tools for the evaluation of the motor system in patients with movement disorders including the electrocutaneous reflex (ECR) and perioral reflexes.

\* Continued:

|                      |                   |      |     |     |       |
|----------------------|-------------------|------|-----|-----|-------|
| Rocco Agostino, M.D. | Visiting Fellow   | HMCS | MNB | DIR | NINDS |
| Helge Topka, M.D.    | Special Volunteer | HMCS | MNB | DIR | NINDS |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02668-05 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trial of Isoniazid for Action Tremor

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Mark Hallett, M.D. Clinical Director OCD ODIR DIR NINDS  
Chief HMCS MNB DIR NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Human Motor Control Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies have shown utility of isoniazid for ameliorating cerebellar postural tremor in patients with multiple sclerosis. Current studies are aimed at identifying whether patients with action tremors of other types are also benefitted. A double-blind placebo-controlled, cross-over trial was completed. Twenty patients have now been studied. Isoniazid does appear to help some of these cases, and the final data analysis is being completed.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02669-05 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Voluntary Movement

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                        |                    |      |      |     |       |
|---------|------------------------|--------------------|------|------|-----|-------|
| P.I.:   | Mark Hallett, M.D.     | Clinical Director  | OCD  | ODIR | DIR | NINDS |
|         |                        | Chief              | HMCS | MNB  | DIR | NINDS |
| Others: | Jerome Sanes, Ph.D.    | Special Expert     | HMCS | MNB  | DIR | NINDS |
|         | Leo Cohen, M.D.        | Visiting Associate | HMCS | MNB  | DIR | NINDS |
|         | Victoria Panzer, Ph.D. | Staff Fellow       | HMCS | MNB  | DIR | NINDS |
|         | Rocco Agostino, M.D.   | Visiting Fellow    | HMCS | MNB  | DIR | NINDS |
|         | Ina Tarkka, Ph.D.      | Volunteer          | HMCS | MNB  | DIR | NINDS |

## COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center  
 Department of Nuclear Medicine, Clinical Center

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Human Motor Control Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.8

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in the Gait Laboratory of the Department of Rehabilitation Medicine have focused on the control of balance. Simultaneous measurement of body angles, foot-floor forces and multiple electromyograms (EMGs) are possible. When standing subjects make rapid arm movements, postural muscle activity precedes activity in arm muscles. Biomechanical analysis shows that the role of this activity is to prevent significant displacement of the center of gravity during the movement and to prevent all but the most minor alterations in angles of the body. We have initiated similar studies in patients with Parkinson's disease and cerebellar disturbances.

Using O-15 labelled water as a marker for cerebral blood flow in positron emission tomography (PET) studies, we have looked for changes in the motor cortex with simple hand movements. We have found an increase in the cerebral blood flow contralateral to voluntary wrist flexion/extension in the motor cortex and supplementary motor cortex.

In patients with Parkinson's disease (PD), there was no delay in initiating movement when the patients were required to remember the location of a targeted movement. Patients with cerebellar hemispheric atrophy failed to improve the performance of sequential movements done with normal vision, whereas patients with olivo-ponto-cerebellar atrophy showed a deficit in performance of sequential movements guided with mirror-vision. Patients with PD performed normally.

In evoked potential studies, we determined that the appropriate interelectrode distances for mapping somatosensory evoked potentials (SEP) is approximately 2.3-3 cm. By using this spatial sampling, we could differentiate maps of SEP obtained to stimulation of different fingers, and cutaneous stimulation of the skin overlying forearm, arm, and shoulder. It was also possible to differentiate sensory maps obtained to stimulation of distal and proximal areas of the leg.

In studies of movement related potentials, we have differentiated components due to motor cortex activation and sensory feedback.

24-MNB/DIR



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02711-04 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|        |                    |                    |      |      |     |       |
|--------|--------------------|--------------------|------|------|-----|-------|
| P.I.:  | Mark Hallett, M.D. | Clinical Director  | OCD  | ODIR | DIR | NINDS |
|        |                    | Chief              | HMCS | MNB  | DIR | NINDS |
| Other: | Leo Cohen, M.D.    | Visiting Scientist | HMCS | MNB  | DIR | NINDS |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Human Motor Control Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.2

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

|  |  |                                      |
|--|--|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors                   |  |                                      |
| <input type="checkbox"/> (a2) Interviews               |  |                                      |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Botulinum toxin injected in small doses directly into muscle binds to the neuromuscular junction and inactivates it for approximately 3 months. This treatment has been demonstrated to be useful for strabismus and blepharospasm, but there has not been a complete understanding of its mechanism of action.

Studies of utility of botulinum toxin have been carried out in spasmodic dysphonia and writer's cramp (and its variants such as pianist's cramp). Treatment appeared effective in both, and we are enlarging our experience with writer's cramp to see if we can determine which patients are more likely to improve. A double-blind trial will be started for writer's cramp.

Studies of physiology of the mode of action have been carried out in spasmodic dysphonia, writer's cramp, blepharospasm and hemifacial spasm. These studies show that the major effect of botulinum toxin is to weaken the muscle that is in spasm. Electromyogram (EMG) studies in writer's cramp, blepharospasm and hemifacial spasm show that spasms continue, but muscles are ineffective. No other changes in physiology were identified.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02712-04-MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Non-invasive Stimulation of Human Central Nervous System

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |                    |      |      |     |       |
|---------|---------------------------|--------------------|------|------|-----|-------|
| P.I.:   | Mark Hallett, M.D.        | Clinical Director  | OCD  | ODIR | DIR | NINDS |
|         |                           | Chief              | HMCS | MNB  | DIR | NINDS |
| Others: | Leo Cohen, M.D.           | Visiting Associate | HMCS | MNB  | DIR | NINDS |
|         | Stefania Bandinelli, M.D. | Guest Researcher   | HMCS | MNB  | DIR | NINDS |
|         | Peter Fuhr, M.D.          | Visiting Fellow    | HMCS | MNB  | DIR | NINDS |
|         | Rocco Agostino, M.D.      | Visiting Fellow    | HMCS | MNB  | DIR | NINDS |
|         | Helge Topka, M.D.         | Special Volunteer  | HMCS | MNB  | DIR | NINDS |

## COOPERATING UNITS (if any)

Speech and Voice Pathology Unit, IRP, NIDCD

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Human Motor Control Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.6

## PROFESSIONAL:

0.5

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recently techniques have become available for the non-invasive stimulation of the human cortex and deep proximal peripheral nerves. Stimulation can be with a high voltage, extremely brief electrical pulse or with magnetic stimulation. One purpose is to use these methods for noninvasive localization of different parts of the human cortex including motor cortex, sensory cortex and language cortex. Another purpose is to study cortical physiology in different disease states.

We have established normative data for our own laboratory for measurement of central motor conduction velocities. We have also found that EEGs do not change after a session of cortical stimulation in normal volunteers and patients, which indicates the safety of the procedure. We have succeeded in mapping the hand, arm, leg and mouth areas of the human motor cortex in normal volunteers and correlating these motor maps with the sensory maps as defined by using somatosensory evoked potentials. At this time we are studying changes in these motor maps in patients with mirror movements, stroke, and with different types of amputations. We have found that patients with congenital mirror movements have a bilateral cortical representation of each hand in the motor cortex. Also, that they have physiologically active and fast conducting connections between the motor cortex and ipsilateral muscles in the upper extremity. We have also studied hemispheric dominance for laryngeal muscles finding that there seems to be bilateral projections from both hemispheres to motoneurons controlling muscles in both sides of the larynx and that stimulation of the left hemisphere activates a larger percentage of the motoneuron pool bilaterally. We have mapped sensory cortex by utilizing the phenomenon of blockage of a cutaneous stimulus. We have also used magnetic stimulation to probe the processes in motor cortex during a reaction time task in patients with Parkinson's disease.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01658-22 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hemispheric Development and Specialization of the Intellectual Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |              |                 |
|---------|-----------------|--------------|-----------------|
| PI:     | P. Fedio, Ph.D. | Psychologist | CNS, MNB, NINDS |
| Others: | E. Mohr, Ph.D.  | Psychologist | CNS, MNB, NINDS |
|         | L. Ryan, M.A.   | Psychologist | CNS, MNB, NINDS |

## COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINDS

## LAB/BRANCH

Medical Neurology, CNP, DIR, NINDS

## SECTION

Clinical Neuropsychology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of chronic cerebral insult and neuropsychiatric disorders were evaluated by a broad range of neuropsychological tests evaluating brain-behavior relations.

Asymptomatic and symptomatic children with Gaucher's and Niemann-Pick disorders were examined with neuropsychological and psychoeducational procedures. Preliminary data indicate that presymptomatic patients manifest selective cognitive deficits, more so with attention and memory tasks. One finding suggests that these children are selectively troubled by visuospatial and constructional deficits, with academic expression in the form of orthographic (spelling) disorders.

Adolescents with obsessive-compulsive features exhibited a configuration of neuropsychological deficits which correlated with ventricular enlargement. Deficits were identified in spatial judgment and spatial learning. An imbalance in the inhibitory functions of frontal and limbic systems may propel obsessive-compulsive behavior.

|   |                      |                                       |
|---|----------------------|---------------------------------------|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                      | PROJECT NUMBER<br>201 NS 01424-23 MNB |
| PERIOD COVERED<br>October 1, 1988 through September 30, 1989  |                      |                                       |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)<br>Behavioral Modulation by the Limbic System in Man  |                      |                                       |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>PI: P. Fedio, Ph.D. Psychologist CNS, MNB, NINDS<br>Others: L. Ryan, M.A. Psychologist CNS, MNB, NINDS<br>D. Ronsaville, Ph.D. Psychologist CNS, MNB, NINDS<br>C. Kufta, M.D. Medical Officer SNB, NINDS<br>S. Sato, M.D. Chief, EEG Lab OCD, DIR, NINDS<br>W. Theodore, M.D. Chief CES, MNB, NINDS<br>A. August, M.A. Psychologist Catholic Univ.<br>C. Cox, M.A. Psychologist Johns Hopkins Univ.  |                      |                                       |
| COOPERATING UNITS (if any)<br>Surgical Neurology Branch, DIR, NINDS<br>Department of Medical Psychology, John Hopkins University, Baltimore, MD<br>Department of Psychology, Catholic University, Washington, D.C.  |                      |                                       |
| LAB/BRANCH<br>Medical Neurology, CNB, DIR, NINDS  |                      |                                       |
| SECTION<br>Clinical Neuropsychology   |                      |                                       |
| INSTITUTE AND LOCATION<br>NINDS, NIH, Bethesda, MD 20892  |                      |                                       |
| TOTAL MAN-YEARS:<br>1.5   | PROFESSIONAL:<br>1.0 | OTHER:<br>0.5                         |
| CHECK APPROPRIATE BOX(ES)<br><input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither<br><input type="checkbox"/> (a1) Minors<br><input type="checkbox"/> (a2) Interviews   |                      |                                       |
| SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)<br><p> <u>Emotional</u> and <u>cognitive</u> characteristics were studied in <u>epileptic patients</u> before and following unilateral left or right <u>temporal lobe</u> resection, and during <u>brain stimulation</u> and <u>intracarotid amytal injection</u>. Physiological events (<u>skin conductance</u>) and <u>electroencephalography (EEG)</u> measures were also monitored during select test performance. The research examined the role of the temporal lobe in establishing <u>limbic sensory</u> associations as a basis for <u>cognition</u> and <u>emotion</u>.         </p> <p>           Left brain stimulation (indwelling flap electrodes) of posterior sites, produced storage and retrieval memory errors with anterior and posterior, temporal sites, respectively. Stimulation of frontal cortex produced defects suggestive of mechanisms that collate immediate and long-term plans. There was a dissociation between <u>aphasia</u> and <u>amnesia</u> with inferior, posterior temporal stimulation, emphasizing the importance of this region to retrieval from <u>episodic memory</u> registers. With right brain stimulation, paralinguistic disturbances were produced in prosody, and there were errors in interpreting ambiguous statements and in pattern discrimination and recognition.         </p> <p>           In affective spheres, despondency followed pharmacological deactivation of the left hemisphere, whereas euphoria accompanied amytal injection into the right internal carotid. The transient mood state was more common for patients with right temporal lesions with late age at onset of seizure disorder. With neuropsychometric procedures, the patients differed along an <u>introversion-extroversion</u> continuum. Patients presenting an aura of fear are more likely to exhibit maladaptive behaviors. These data suggest that unilateral temporal lobe injury disrupts the normal linkage of cognitive-affective associations mediated by frontal-limbic interaction.         </p> |                      |                                       |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01245-24 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Fedio, Ph.D.

Psychologist

CNS, MNB NINDS

Others: C. Kufta, M.D.

Medical Officer

SNB, NINDS

S. Sato, M.D.

Chief, EEG Lab

OCD, DIR, NINDS

B. Smith, M.D.

Psychologist

University of MD

## COOPERATING UNITS (if any)

Surgical Neurology Branch, DIR, NINDS

Department of Psychology, University of MD., College Park, MD

## LAB/BRANCH

Medical Neurology, CNP, DIR, NINDS

## SECTION

Clinical Neuropsychology

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Perception and judgment with cognitive and emotional tasks were monitored by electroencephalography (EEG) spectral activity recorded from left and right brain regions, of patients following unilateral temporal lobectomy. EEG disturbances in brain-behavior relations in neuropsychiatric patients were also evaluated, relating left and right brain dysfunctioning to maladaptive ideative and emotional reactions, respectively.

With temporal lobectomy patients, preliminary results indicate that electrography shifts in frequency-amplitude differed in that right temporal patients showed greater responsibility to pleasant and horrific materials, less so while applying cognitive strategies to deal with imaginary emotionally charged situations. The converse was true with left temporal patients who generated greater activity while intellectually resolving emotional tasks. These data underscore the dual cognitive and emotional roles of the limbic system in tempering human behavior.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00200-35 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Cognitive and Emotional Profile of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                 |              |                   |
|---------|-----------------|--------------|-------------------|
| PI:     | P. Fedio, Ph.D. | Psychologist | CNS, MNB, NINDS   |
|         | E. Mohr, Ph.D.  | Psychologist | CNS, MNB, NINDS   |
| Others: | T. Chase, M.D.  | Neurologist  | Chief, ETB, NINDS |
|         | L. Ryan, M.A.   | Psychologist | CNS, MNB, NINDS   |

## COOPERATING UNITS (if any)

Medical Neurology, CNP, DIR, NINDS  
Experimental Therapeutics Branch, DIR, NINDS

## LAB/BRANCH

Clinical Neuropsychology

## SECTION

NINDS, NIH, Bethesda, MD 20892

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A neuropsychological profile was plotted for individuals with Alzheimer's (AD), Huntington's (HD), or Parkinson's (PD) disease. The evaluations extended into memory, learning and perception, utilizing standard and experimental tasks, also establishing normative references for functional changes accompanying the aging processes.

The results revealed common as well as specific deficits, implicating involvement of different brain structures. Specifically, AD is accompanied by marked deficits in selective attention and episodic memory, and visuospatial disturbances; there were few qualitative differences between demented and age-matched subjects. These data indicate that Alzheimer's patients may be unable to encode material.

AD and HD patients showed pronounced but dissimilar deficits with visuospatial and constructional tasks. The behavioral data extend neuropathologic impressions of degeneration of the frontal striatal system in HD and temporo-parietal, cortical involvement in AD.

With PD, performance decrements were less prominent and many patients continue to function at an unimpaired level; dysfunctioning varied in relation to complexity and executive requirements, which aligned strongly with fronto-striatal changes. Unlike HD, PD patients usually showed fewer behavioral and personality changes; emotional expression was not one of disinhibition.



|   |                       |  |
|---|-----------------------|--|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                       | <b>PROJECT NUMBER</b><br><br>Z01 NS 02678-05 MNB |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989 *   |                       |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Studies of Human Epileptic Focus  |                       |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |                       |  |
| PI:   | N. Suzan Nadi, Ph. D. | Senior Staff Fellow    NEXS    MNB    NINDS      |
| OTHERS:   | Manuela Pintor, M.D.  | Visiting Associate    NEXS    MNB    NINDS       |
|   | Ivan Mefford, Ph.D.   | Special Expert    SCP    LCS    NIMH             |
|   | Karen Wayns           | Bio Lab Tech    NEXS    MNB    NINDS             |
|   | Helen Pan, Ph.D.      | Staff Fellow    PNS    ETB    NINDS              |
|   | J. Walters, Ph.D.     | Chief    PNS    ETB    NINDS                     |
| <b>COOPERATING UNITS</b> (if any)<br>University of Tennessee (A. Wyler); Yale University (D. Morrow); Hebrew University (D. Lichstein)  |                       |  |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR   |                       |  |
| <b>SECTION</b><br>Neuronal Excitability Section   |                       |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892   |                       |  |
| <b>TOTAL MAN-YEARS:</b> 1.6   |                       | <b>PROFESSIONAL:</b> 0.6                         |
|   |                       | <b>OTHER:</b> 1.0                                |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>   |                       |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p>           The nature of the chemical alterations in the <u>human epileptic cortex</u> were investigated further using <u>in vitro</u> slice techniques and <u>autoradiography</u>. The <u>in vitro</u> slices, and the addition of <u>norepinephrine</u>, <u>glutamate</u>, <u>NMDA</u>, and <u>somatostatin</u>, were used individually or in combination in order to determine whether the epileptic focus had a different responsiveness at the level of <u>cyclic nucleotides</u> and <u>inositol phosphate</u>. The responsiveness of the epileptic tissue under <u>in vitro</u> conditions was not significantly different from the surrounding less epileptic tissue. PET scan studies have indicated that the binding of the mu opioid receptor was slightly elevated in the spiking epileptic cortex when compared to the non-spiking cortex. To investigate this finding further, sections of the spiking and nonspiking epileptic cortex were exposed to [3-H]-DAGO, a selective <u>mu opiate receptor</u> ligand, and adjacent sections to [3-H]-DADLE, a <u>mu and delta receptor</u> ligand. The major findings of the study are that there are no significant differences for the number of receptor sites or their affinity in the spiking versus nonspiking cortex. The distribution of the mu and delta receptors was observed to be different. In the spiking and nonspiking cortex, the mu receptors were in a heterogeneous manner with the peak layers found in two bands at 1/3 and 1/2 the distance between the meninges and the white matter. In the same tissues, the delta receptor was homogeneously distributed but did have a gradient with the highest number of receptors located in the outermost layer of the cortex. The number of <u>TCP receptors</u> in the spiking versus nonspiking cortex was also investigated using [3-H]-TCP. The findings were that there were no receptor number or affinity changes when the spiking and nonspiking tissues were compared. The distribution of TCP receptors was similar in the two tissues and had a gradient with the highest number of receptors localized in the outermost layers of the cortex.         </p> |                       |  |
| * This project is terminated 9/30/89.   |                       |  |

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| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |  | <b>PROJECT NUMBER</b><br><br>Z01 NS 02713-04 MNB |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989 *   |  |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Molecular Biologic Basis of Kindling  |  |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |  |  |
| PI:   | N. Suzan Nadi, PH.D.    Senior Staff Fellow    NEXS    MNB    NINDS  |  |
| OTHERS:   | Joan Schwartz, Ph.D.    Research Chemist    NPS    CNB    NINDS<br>H. Shinoda, M.D.    Visiting Fellow    NPS    CNB    NINDS<br>C. Cosi, Ph.D.    Visiting Fellow    NPS    CNB    NINDS<br>Manuela Pintor, M.D.    Visiting Fellow    NEXS    MNB    NINDS<br>Karen Wayns    Bio. Lab. Tech.    NEXS    MNB    NINDS |  |
| <b>COOPERATING UNITS</b> (if any)<br>University of Tennessee (A. Wyler); Surgical Neurology Branch, NINDS, (C. Kufta); UCLA (A. Tobin)  |  |  |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR   |  |  |
| <b>SECTION</b><br>Neuronal Excitability Section   |  |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892   |  |  |
| TOTAL MAN-YEARS:  | 0.8  | PROFESSIONAL: 0.6    OTHER: 0.2                  |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>   |  |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p>Previous work from our laboratory has demonstrated a time dependent change in the levels of <u>somatostatin</u> in the cortex and hippocampus of the kindled rat brain. In addition, the activity of tyrosine hydroxylase is increased immediately following a seizure. <u>Glutamate decarboxylase</u> levels were not found to be changed in any stage of the kindled rat brain. In view of the fact that some regulatory DNA such as <u>c-fos</u> are altered in the seizure state, it was of interest to undertake a study of the expression of the mRNA for <u>somatostatin</u>, <u>tyrosine hydroxylase</u> and glutamate decarboxylase and their correlation with the expression of the c-fos gene to determine if c-fos regulates the levels of expression of the above-mentioned compounds.</p> <p>Currently the above studies are being expanded to incorporate the time course of the development of mRNA alterations and additional investigations of blocking of <u>seizures</u> on the expression of the mRNA are being undertaken. Long-term studies include the <u>in situ hybridization</u> studies of the human epileptic focus and comparing it to the nonfocal tissue from the same patient.</p> <p>These studies will help determine the phase of the seizure progression when the mRNA changes occur. Such observations will help understand how the expression of genes is influenced in the situation where increased spiking activity occurs in the brain. Such an understanding of the kindled brain may help in elucidating the long-term changes which occur in the human epileptic focus.</p> <p>Similarly studies are ongoing to correlate the changes in amino acid transmitters, <u>neuropeptides</u>, <u>enzymes</u> and <u>receptors</u> with the development of seizures.</p> |  |  |
| * This project is terminated 9/30/89.   |  |  |

|   |                          |  |
|---|--------------------------|--|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                          | <b>PROJECT NUMBER</b><br><br>Z01 NS 02736-03 MNB |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989 *   |                          |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Mechanisms of Kindled Seizure Suppression by Cysteamine   |                          |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>PI:                      N. Suzan Nadi, Ph.D.                      Senior Staff Fellow                      NEXS                      MNB                      NINDS  |                          |  |
|   |                          |  |
| <b>COOPERATING UNITS</b> (if any)   |                          |  |
|   |                          |  |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR   |                          |  |
| <b>SECTION</b><br>Neuronal Excitability Section   |                          |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892   |                          |  |
| <b>TOTAL MAN-YEARS:</b> 0.1   | <b>PROFESSIONAL:</b> 0.1 | <b>OTHER:</b> 0                                  |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>   |                          |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p>Cysteamine has been shown to suppress kindled seizures at doses from 90 mg/kg to 300 mg/kg when given to rats kindled to stage V. This project will involve the careful evaluation of the alterations in brain chemistry and the onset of the suppression of seizures in order to better understand the mechanism of action of cysteamine.</p> <p>The aims of the present project are to better understand the mechanisms by which cysteamine eliminates seizures. Rats which are kindled and sham operated will receive a single intraperitoneal injection of cysteamine (200 mg/kg). Following the administration of the drug, the animals will be observed for behavioral changes as well as seizure suppression. The rats will be killed at known time intervals following cysteamine administration, the brain removed, and the cortex, cerebellum, midbrain, pons-medulla, and hippocampus will be dissected. These tissues will be extracted and <u>evaluated for peptide, amino acid, receptor, and catecholamine levels</u>. The correlation of the chemical changes to the seizure suppression may allow the identification of one chemical alteration with a decrease in seizure activity. The next step in the study will be to determine if antagonists of the compound will also suppress seizures. Studies at present from our laboratory as well as others show that both somatostatin and norepinephrine are decreased as a result of cysteamine administration.</p> <p>The results of the cysteamine experiments have shown that following administration of the drug, the suppression of seizures occurs not at the point where somatostatin is the lowest, but at a point where the levels of somatostatin are the closest to the control levels. These observations suggest that the suppression of the seizures following administration of the drug may be due to a receptor resensitization rather than the decrease of the somatostatin itself. Studies have shown that the decrease of somatostatin closely parallels the block of seizures, and its increase parallels the return of seizures. These observations suggest that the alteration in the levels of somatostatin may play a therapeutic role in seizures.</p> <p>*This project is terminated 9/30/89.</p> |                          |  |
| 33-MNB/DIR  |                          |  |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02737-03 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989 \*

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transplant of GABAergic Neurons into the Substantia Nigra of Kindled Rats

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N.Suzan Nadi, Ph. D. Senior Staff Fellow NEXS MNB NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Neuronal Excitability Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.2

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project will investigate the effect of transplanting neuronal suspensions from the cerebellum of 16 day old rat embryos into the substantia nigra of adult kindled rats. The hypothesis is that the substantia nigra is involved in the control of spread of seizures and the addition of gamma amino butyric acid (GABA)ergic neurons to the nigra will prevent the seizures.

GABAergic neurons are obtained by gentle trypsinization of the cerebellum of 16 days old embryos. These cells are then transplanted into the substantia nigra using a Harvard pump. The origin of the cells will be determined by staining with the glutamate decarboxylase (GAD) antibody as well as cerebellum specific antibodies.

Our most recent results show at a light microscopic level a fine network of cells at the area of transplant in the nigra. These cells are elongated in shape with extensive processes. They have failed to stain with GAD antibody, but other GABAergic neurons in the area have not stained either. We are currently investigating the reasons behind this. Preliminary work has shown that kindling disappears for 2 weeks after transplant but then returns. The reasons for these findings are being investigated. The substantia nigra has been shown to be a controlling point of seizure spread by numerous investigators. The current study will attempt to demonstrate whether transplants of GABAergic neurons from rat embryo cerebellum will block the spread of seizures.

The results of these studies were largely negative. The reasons for these findings could be multiple, among them the failure to be able to grow the GABAergic neurons and to form synapses. The conclusions of this study are that further basic knowledge of the neurobiology of brain transplants is needed before their usefulness in neurologic diseases can be evaluated.

\*This project is terminated 9/30/89.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological Studies of Ion Channels in Cultured Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Rogawski, M.D., Ph.D. Senior Staff Fellow NEXS MNB NINDS

Others: Setsuo Suzuki, M.D. Visiting Fellow NEXS MNB NINDS  
 Dora M.T. Politi, M.D. Special Volunteer NEXS MNB NINDS  
 Norman Herschkowitz, M.D., Ph.D. NRC Fellow NEXS MNB NINDS  
 J.M.H. Ffrench-Mullen, Ph.D. NRC Fellow LNP NINDS

## COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NINDS

## LAB/BRANCH

Medical Neurology Branch, CNP, DIR

## SECTION

Neuronal Excitability Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.5

## PROFESSIONAL:

3.5

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Drug interactions with voltage-dependent K channels and N-methyl-D-aspartate (NMDA) receptor coupled cation channels were studied in cultured hippocampal neurons using whole-cell voltage-clamp and single channel recording techniques. The aim of this work was to develop new strategies for the rational development of antiepileptic drugs based upon their interaction with neuronal ion channel systems. Work was focused in three areas: (1) phencyclidine (PCP) related drugs, (2) antagonists of the glycine modulatory site on the NMDA receptor, and (3) K channel activator drugs. PCP (0.5 - 1000  $\mu$ M) caused a reduction in the maximum conductance of the slowly activating K current I-K in hippocampal neurons [IC-50( + 30 mV), 22  $\mu$ M] without altering its voltage-dependency. The PCP block of I-K diminished at depolarized potentials. Analysis according to the scheme of Woodhull suggested that block occurs via binding to an acceptor site (presumably within the channel pore) that senses 40-50% of the transmembrane electrostatic field. PCP reduced inward current responses induced by the excitatory amino acid agonist NMDA at substantially lower concentrations than those required for its effects on K channels [IC-50(-60mV), 0.45  $\mu$ M]. The affinity of PCP for its acceptor site on I-K channels is 13 times lower than its affinity for NMDA-receptor associated channels. Therefore, at low doses, the behavioral effects of the drug are more likely to result from an interaction with NMDA receptor-channels than voltage-dependent K channels. Another approach to diminishing NMDA-receptor mediated excitation is blockade of the glycine modulatory site on the NMDA-receptor channel complex. Using the whole-cell voltage-clamp technique, we demonstrated that the glycine-analog cycloleucine is a competitive antagonist at the glycine site. In the presence of 1  $\mu$ M glycine, cycloleucine caused a reversible, dose-dependent inhibition of NMDA responses with an IC-50 of 24  $\mu$ M. An increase in glycine to 100  $\mu$ M resulted in a shift to the right of the cycloleucine concentration-effect curve (IC-50, 1.4  $\mu$ M). Cycloleucine failed to affect kainic acid and quisqualic acid evoked currents at concentrations which inhibited NMDA responses. As an alternative anticonvulsant strategy, we explored means of pharmacologically activating voltage-dependent K channels. We observed that the antihypertensive cromakalim (BRL 34915) causes a dramatic increase in the slowly activating, sustained outward current in cultured hippocampal neurons. Drugs like cromakalim which activate K channels in CNS neurons have potential as anticonvulsants.



|  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
|--|----------------------------------|---|------|-----|----------------------------------|---------------------|------|-----|-------|---------|----------------------|----------------|------|-----|-------|--|-------------------------|--------------|--|-----|-------|--|-------------------|-------|--|-----|-------|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                                  | <b>PROJECT NUMBER</b><br><br>Z01 NS 02733-03 MNB  |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>TITLE OF PROJECT</b> <small>(80 characters or less. Title must fit on one line between the borders.)</small><br>Excitability Properties of Enzymatically Dissociated CNS Neurons  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>PRINCIPAL INVESTIGATOR</b> <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small><br><table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Michael A. Rogawski, M.D., Ph.D.</td> <td style="width: 20%;">Senior Staff Fellow</td> <td style="width: 10%;">NEXS</td> <td style="width: 10%;">MNB</td> <td style="width: 10%;">NINDS</td> </tr> <tr> <td>Others:</td> <td>Setsumi Suzuki, M.D.</td> <td>Fogarty Fellow</td> <td>NEXS</td> <td>MNB</td> <td>NINDS</td> </tr> <tr> <td></td> <td>Michael Weinstein, M.D.</td> <td>Staff Fellow</td> <td></td> <td>MRB</td> <td>NIDDK</td> </tr> <tr> <td></td> <td>John Rinzel, M.D.</td> <td>Chief</td> <td></td> <td>MRB</td> <td>NIDDK</td> </tr> </table>   |                                  |   |      | PI: | Michael A. Rogawski, M.D., Ph.D. | Senior Staff Fellow | NEXS | MNB | NINDS | Others: | Setsumi Suzuki, M.D. | Fogarty Fellow | NEXS | MNB | NINDS |  | Michael Weinstein, M.D. | Staff Fellow |  | MRB | NIDDK |  | John Rinzel, M.D. | Chief |  | MRB | NIDDK |
| PI:  | Michael A. Rogawski, M.D., Ph.D. | Senior Staff Fellow   | NEXS | MNB | NINDS                            |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| Others:  | Setsumi Suzuki, M.D.             | Fogarty Fellow  | NEXS | MNB | NINDS                            |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
|  | Michael Weinstein, M.D.          | Staff Fellow  |      | MRB | NIDDK                            |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
|  | John Rinzel, M.D.                | Chief   |      | MRB | NIDDK                            |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>COOPERATING UNITS</b> <small>(if any)</small><br>Mathematical Research Branch, NIDDK  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>SECTION</b><br>Neuronal Excitability Section  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>TOTAL MAN-YEARS:</b><br><div style="display: flex; justify-content: space-between; width: 100%;"> <span>1.0</span> </div>   |                                  | <b>PROFESSIONAL:</b><br><div style="display: flex; justify-content: space-between; width: 100%;"> <span>1.0</span> </div> |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
|  |                                  | <b>OTHER:</b><br><div style="display: flex; justify-content: space-between; width: 100%;"> <span>0</span> </div>          |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>  |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |
| <b>SUMMARY OF WORK</b> <small>(Use standard unreduced type. Do not exceed the space provided.)</small><br><p>Electrophysiological techniques were used to characterize the ionic channels of neurons isolated from slices of the adult <u>guinea pig thalamus</u>. Thalamic neurons undergo a shift from tonic to phasic (burst) firing upon hyperpolarization. This state transition results from deinactivation of a regenerative depolarizing event referred to as the <u>low-threshold spike (LTS)</u>. Isolated thalamic (<u>dorsal lateral geniculate</u>) neurons exhibited low-threshold spikes that could be blocked by low concentrations of <u>nickel</u> but were unaffected by the dihydropyridine <u>nimodipine</u>. <u>Whole cell voltage-clamp recordings</u> from these cells demonstrated a low-threshold, rapidly inactivating (T) calcium current that manifested similar voltage-dependency and time course as the low threshold spike. Like low threshold spikes, the <u>T-type calcium</u> current was eliminated by nickel but was unaffected by nimodipine. In thalamic neurons, T-type calcium channels underlie the low threshold spike, and therefore play a critical role in regulating the firing pattern of these cells. Hodgkin-Huxley modeling of the LTS indicated that its shape can be accounted for almost entirely by the intrinsic properties of T-type voltage-dependent calcium channels. Burst firing mediated by the LTS is critical to the generation of <u>absence seizures</u> and drugs which specifically block the LTS (T-type calcium channels) prevent absence seizures. Therefore, isolated thalamic neurons are likely to be a useful experimental system for the evaluation of potential new anti-absence drugs.</p> |                                  |   |      |     |                                  |                     |      |     |       |         |                      |                |      |     |       |  |                         |              |  |     |       |  |                   |       |  |     |       |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 NS 02772-02 MNB

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Phencyclidine Analogs as Anticonvulsants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                                 |                           |      |     |       |
|---------|---------------------------------|---------------------------|------|-----|-------|
| PI:     | Michael A. Rogawski, M.D., Ph.D | Senior Staff Fellow       | NEXS | MNB | NINDS |
| Others: | Shun-Ichi Yamaguchi, Ph.D       | Psychologist              | NEXS | MNB | NINDS |
|         | Andrew Thurkoff, Ph.D.          | NRSA Fellow               |      | MC  | NIDDK |
|         | Brian de Costa, Ph.D.           | Visiting fellow           |      | MC  | NIDDK |
|         | Kenner C. Rice, Ph.D.           | Chief                     |      | MC  | NIDDK |
|         | Arthur E. Jacobson, Ph.D.       | Senior Research Scientist |      | MC  | NIDDK |

COOPERATING UNITS (if any)

Laboratory of Medicinal Chemistry, NIDDK

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, Bethesda, MD 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work was continued on the evaluation of a series of phencyclidine (PCP) analogs for anticonvulsant activity in several animal seizure models. The dissociative anesthetic PCP is a powerful anticonvulsant in a wide variety of animal seizure models. However, undesirable side effects which occur in the same dosage range as seizure protection limit its practical usefulness in the treatment of seizure disorders. Despite its unfavorable therapeutic index, PCP can be considered a prototype anticonvulsant upon which to base the design of less toxic and potentially more clinically useful drugs. We have examined the anticonvulsant activity of more than 40 PCP analogs in an attempt to obtain compounds with enhanced anticonvulsant activity relative to their neurotoxic side effects. Drugs were screened for anticonvulsant activity in mice with the maximal electroshock (MES) test and by administration of the chemoconvulsants pentylene-tetrazol (PTZ) and N-methyl-D-aspartate (NMDA). PCP had approximately equal potency in the MES test and in a motor toxicity test so that its "therapeutic index" (TI; ratio of dose causing toxicity in 50% of animals) was about 1. We also observed that PCP and certain of the PCP-related compounds noted above were highly potent in preventing NMDA induced seizures, but were weak or ineffective against PTZ induced convulsions. The most favorable analogs were derivatives of 1-phenylcyclohexylamine modified by (a) certain stereochemically orientated cyclohexane ring methyl substituents, (b) ortho substituents on the phenyl ring, and (c) contraction of the cyclohexane ring. In addition, we found that conformational restriction of the PCP molecule as in 1, 1-pentamethylene-tetrahydroisoquinoline resulted in an improved TI. Studies were also begun on a series of compounds related to the potent NMDA-receptor channel blocker MK-801. Of particular interest is S-aminocarbonyl-5H-dibenzo [a,d] cyclohepten-5, 10-mimine which was a very potent anticonvulsant in MES test (ED-50, 8-9 mg/kg) and showed a six-fold improvement in therapeutic index compared with PCP.

|  |                         |                          |  |                   |       |
|--|-------------------------|--------------------------|--|-------------------|-------|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                         |                          | <b>PROJECT NUMBER</b><br>Z01 NS 02792-01 MNB |                   |       |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |                         |                          |  |                   |       |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Neuropsychological Investigations of Human Cognition and Mood State  |                         |                          |  |                   |       |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)   |                         |                          |  |                   |       |
| PI:  | Jordan Grafman, Ph.D.   | Psychologist             | CNU  | MNB               | NINDS |
| Others :   | Ray Johnson, Jr., Ph.D. | Psychologist             | CNU  | MNB               | NINDS |
|  | Rhonda Friedman, Ph.D.  | Psychologist             | CNU  | MNB               | NINDS |
|  | Francois Lalonde, Ph.D. | Psychologist             | CNU  | MNB               | NINDS |
|  | Mark Hallett, M.D.      | Chief                    |  | MNB               | NINDS |
| <b>COOPERATING UNITS</b> (if any)  |                         |                          |  |                   |       |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR  |                         |                          |  |                   |       |
| <b>SECTION</b><br>Cognitive Neuroscience Unit, Office of The Chief   |                         |                          |  |                   |       |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892  |                         |                          |  |                   |       |
| <b>TOTAL MAN-YEARS:</b> 0.4  |                         | <b>PROFESSIONAL:</b> 0.2 |  | <b>OTHER:</b> 0.2 |       |
| <b>CHECK APPROPRIATE BOX(ES)</b>   |                         |                          |  |                   |       |
| <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither   |                         |                          |  |                   |       |
| <input type="checkbox"/> (a1) Minors   |                         |                          |  |                   |       |
| <input type="checkbox"/> (a2) Interviews   |                         |                          |  |                   |       |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p>Current studies in the Cognitive Neuroscience Unit focus on <u>amnesia</u>, <u>thinking</u>, <u>neurolinguistics</u>, <u>event-related evoked potentials</u>, <u>social cognition</u>, and <u>visual processing</u>. Both single-case and group design studies are used. Normal controls, inpatients and outpatients are evaluated. <u>Memory</u> is studied in experiments focusing on implicit and explicit retrieval, priming, autobiographical recall, -discourse processing, naming and word retrieval, and categorization tasks. <u>Reasoning</u> and <u>problem-solving</u> are studied in experiments focusing on planning, analogical thinking, and schema organization. <u>Dyslexia</u>, <u>dysgraphia</u>, and <u>dysnomia</u>, are studied in experiments focusing on single word reading and writing, lexical decision, associative and semantic priming, and similar tasks. <u>Event-related evoked potentials</u> are measured for latency, amplitude, and distribution and used as a physiological indice of information-processing stages. <u>Emotions</u>, <u>impression</u> and <u>preference formation</u>, and social judgment are studied in experiments focusing on judgment of interpersonal behavior, aesthetics, and mood state. Finally, visual information processing is studied beginning with experiments examining spatial frequency contrast-sensitivity, further experiments on object recognition, and other research requiring visual categorization. Although developing theoretically valid and testable models of cognitive processing is the primary aim of the Unit, there is also a strong effort to relate the profile of cognitive deficits in patients to lesion location in order to topographically map the components of cognitive processing to brain regions and systems. <u>Pharmacologic challenge</u> studies (e.g., using anticholinergics) are planned to evaluate the dissociability of hypothesized components of memory processing. <u>PET scan studies</u> are planned to examine whether familiar and unfamiliar faces are processed in a different brain location from real and non-objects and forms.</p> |                         |                          |  |                   |       |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02793-01 MNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amnesia and Cognitive Neuroscience

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                         |                 |     |     |       |
|---------|-------------------------|-----------------|-----|-----|-------|
| PI:     | Jordan Grafman, Ph.D.   | Psychologist    | CNU | MNB | NINDS |
| Others: | Francois Lalonde, Ph.D. | Psychologist    | CNU | MNB | NINDS |
|         | Ray Johnson, Jr., Ph.D. | Special Expert  | CNU | MNB | NINDS |
|         | Rhonda Friedman, Ph.D.  | Special Expert  | CNU | MNB | NINDS |
|         | Jeffrey Hadley, Ph.D.   | IRTA            | CNU | MNB | NINDS |
|         | Angela Sirigu, Ph.D.    | Visiting Fellow | CNU | MNB | NINDS |

\*

## COOPERATING UNITS (if any)

Walter Reed Army Medical Center, Wash, DC; National Naval Medical Center, Bethesda, MD; Centre Paul Broca, Paris, France; Hopital Salpetriere, Paris, France; Hospital Clinicas, Montevideo, Uruguay; \*\*

## LAB/BRANCH

Medical Neuroscience Branch, CNP, DIR

## SECTION

Cognitive Neuroscience Unit, Office of The Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

0.4

## PROFESSIONAL:

0.2

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Memory and cognition are studied in experiments focusing on representational knowledge, working memory, priming, procedural learning, number processing and calculation, autobiographical memory, naming, and categorization. Normal subjects and patients with progressive dementia, focal lesions, and psychiatric disorders are studied. New studies focusing on the composition of mental structures in the frontal lobes have just begun.

## \*Continued:

|                     |   |
|---------------------|---|
| A. Salazar, M.D.    | Department of Neurology, Walter Reed Army Med. Ctr.         |
| S. Rao, Ph.D.       | Dept. of Neurology, Medical College of Wisconsin            |
| F. Boller, Ph.D.    | INSERM U. 324 Centre Paul Broca, Paris, France              |
| Y. Agid, M. D.      | INSERM U. 289 Hopital Salpetriere, Paris, France            |
| J. Sergent, Ph.D.   | Department of Neurology, Montreal Neurological Institute    |
| C. Chouza, M.D.     | Neurology Institute, Hospital Clinicas, Montevideo, Uruguay |
| D. Hermann, Ph.D.   | National Institute of Mental Health                         |
| J. Hallenbeck, M.D. | MD Department of Neurology, National Naval Medical Center   |

\*\* Medical College of Wisconsin, Milwaukee, Wisconsin; Montreal Neurological Institute, Montreal, Canada; National Institute of Mental Health, NIH.



|   |                         |  |
|---|-------------------------|--|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                         | <b>PROJECT NUMBER</b><br>Z01 NS 02794-01 MNB |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989   |                         |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Event-Related Potential Studies of Normal and Abnormal Cognitive Processing   |                         |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |                         |  |
| PI:   | Ray Johnson, Jr., Ph.D. | Psychologist CNU MNB DIR NINDS               |
| Others:   | Marten Scheffers, Ph.D. | Psychologist CNU MNB DIR NINDS               |
|   | Jordan Grafman, Ph.D.   | Chief CNU MNB DIR NINDS                      |
|   | Christine Olio, Ph.D.   | Psychologist LCS DIR NIMH                    |
|   | Daniel Ruchkin, Ph.D.   | Elec. Engineer U. of MD School of Medicine   |
|   | Wolfgang Miltner, Ph.D. | Psychologist U. of Tuebingen, West Germany   |
| <b>COOPERATING UNITS</b> (if any)<br>National Institute of Mental Health, University of Maryland School of Medicine, University of Tuebingen, West Germany  |                         |  |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, CNP, DIR   |                         |  |
| <b>SECTION</b><br>Cognitive Neuroscience Unit, Office of The Chief  |                         |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892   |                         |  |
| TOTAL MAN-YEARS:  | 2.0                     | PROFESSIONAL: 2.0      OTHER: 0              |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>   |                         |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p> <u>Information processing</u> was monitored by averaged <u>event-related brain potentials</u> (ERP) from a multi-electrode scalp montage during a variety of <u>cognitive</u> tasks in normal subjects, patients with unilateral temporal lobectomy, patients with cortical and subcortical <u>dementias</u>, and patients with other <u>neuropsychiatric disorders</u>. In <u>temporal lobectomy patients</u>, a double dissociation was found in the patients' responses to auditory and visual material. Moreover, there were no consistent hemispheric asymmetries which distinguished the left from the right temporal lobectomy patients, or either group from normal controls. These data discount the hypothesis that medial temporal structures, including the <u>hippocampus</u>, serve as the sole <u>generator</u> of the P300 component of the ERP.         </p> <p>           ERP studies of dementia are continuing for patients with <u>Alzheimer's disease</u> and <u>HIV</u> infections while data collection is complete on patients with <u>progressive supranuclear palsy</u>. Preliminary results suggest that "<u>subcortical</u>" <u>dementias</u> are characterized by abnormalities in both the early (sensory) and late (cognitive) component of the ERP, while "<u>cortical</u>" <u>dementias</u> are characterized by abnormalities exclusively in the late components of the ERP.         </p> <p>           Patient and control data have been used to validate the predictions of a previously hypothesized model of the variables controlling P300 amplitude. In addition, such data have revealed that, contrary to the widely accepted conceptualization, the P300 is a modality-dependent component and its amplitude represents the sum of a number of distinct neural generators.         </p> |                         |  |



## NOTICE OF INTRAMURAL RESEARCH PROJECT

201-NS-02038-17-MNB\*

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Combined Clinical, Viral and Immunological Studies of Neuromuscular Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                        |                   |                        |
|---------|------------------------|-------------------|------------------------|
| PI:     | M.C. Dalakas, M.D.     | Medical Officer   | MNB, DIR, NINDS        |
| Others: | M. Hallett, M.D.       | Clinical Director | OD, DIR, NINDS         |
|         | R. Quarles, Ph.D.      | Biochemist        | DMN, DIR, NINDS        |
|         | P. Plotz, M.D.         | Rheumatologist    | ARB, DIR, NIMSD        |
|         | G.H. Pezeshkpour, M.D. | Neuropathologist  | AFIP, Washington, D.C. |
|         | G. Di Chiro, M.D.      | Neuroradiologist  | OD, DIR, NINDS         |
|         | R. Podolsky, Ph.D.     | Biochemist        | ARB, DIR, NIMSD        |
|         | W.A. Gahl, M.D.        | Medical Officer   | HGB, DIR, NICHD        |

## COOPERATING UNITS (if any)

AFIP, Washington, D.C.

## LAB/BRANCH

Medical Neurology Branch

## SECTION

Neuromuscular Diseases Unit, Office of The Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.0

## OTHER:

.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) of chronic diseases of the peripheral and central nervous system. Current studies include amyotrophic lateral sclerosis (ALS), polymyositis/dermatomyositis, post-polio syndrome, demyelinating polyneuropathies, neuromuscular diseases associated with HIV infection, certain metabolic muscle diseases and Duchenne's muscular dystrophy. The post-polio syndrome, a neuromuscular disease that occurs in patients who have had poliomyelitis at an early age, has been clinically defined. The pathogenesis of this syndrome has been investigated with a series of electrophysiological, virological, immunological, and histological studies, and its relevance to other motor neuron diseases has been examined. The spectrum of neuromuscular disorders associated with HIV infection has been studied and the role of the virus as the cause of neuropathies or myopathies in HIV-positive patients was investigated with a variety of immunocytochemical studies and in situ hybridization. The neuromuscular complications of antiretroviral drugs AZT and DDC, (one causing myopathy and the other a painful neuropathy), were studied and factors that could limit their neurotoxicity were identified. Patients with polymyositis are studied and the muscle changes before and after a randomized double-blind controlled study with intravenous methotrexate, azathioprine or plasmapheresis/lymphocytopheresis are investigated. The force of individual (skinned) muscle fibers from the muscle biopsies of patients with Duchenne's muscular dystrophy was measured and correlated with the biochemical composition of the contractile muscle proteins. We have found that in spite of the absence of dystrophin, muscle fibers in Duchenne's dystrophy generate normal force. The metabolic activity of the cortex in ALS patients was studied using positron emission tomography (PET) scan and 18FDG and the metabolic abnormalities were correlated with the morphological changes in the neurons on brain tissues obtained at autopsy. A variety of neuromuscular diseases associated with nephropathic cystinosis and renal Fanconi syndrome were studied morphologically and biochemically. A myopathy with lipid or cystine storage in the muscle was found prompting a therapeutic trial with carnitine or cysteamine.

\*Transferred from IDB on 10/1/88.

41-MNB/DIR

|   |   |   |
|---|---|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |   | <b>PROJECT NUMBER</b><br><br>ZO1 NS 02531-08 MNB*   |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989   |   |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Studies in Neuromuscular and CNS Diseases and Their Experimental Models   |   |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  |   |   |
| PI: M.C. Dalakas, M.D.<br>Others: M. Gravel, Ph.D.<br>R. Quarles, Ph.D.<br>M. Mutchnick, M.D.<br>I. Illia, M.D., Ph.D.<br>F. Zhao, M.D.<br>S. Koenig, M.D.  | Medical Officer<br>Research Microbiologist<br>Biochemist<br>Gastroenterologist<br>Neurologist<br>Neurologist<br>Research Immunologist | MNB, DIR, NINDS<br>LCNSS, DIR, NINDS<br>DMN, DIR, NINDS<br>Detroit, MI<br>MNB, DIR, NINDS<br>MNB, DIR, NINDS<br>LIR, DIR, NIAID |
| <b>COOPERATING UNITS</b> (if any)<br>Department of Gastroenterology, Wayne State University, Detroit, MI  |   |   |
| <b>LAB/BRANCH</b><br>Medical Neurology Branch, DIR  |   |   |
| <b>SECTION</b><br>Neuromuscular Diseases Unit, Office of The Chief  |   |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892   |   |   |
| <b>TOTAL MAN-YEARS:</b> 1.5   | <b>PROFESSIONAL:</b> 1.0  | <b>OTHER:</b> .5  |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>  |   |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p> <u>Immunocytochemical</u> studies were conducted using specific antibodies to <u>thymic</u> peptides to investigate the interaction of the <u>immune system</u> with the <u>central and peripheral nervous systems</u>. <u>Thymosin beta 4</u>, an immunomodulating thymic polypeptide, was found to be a common antigen shared by macrophages, dendritic lymphoid cells and the myelin producing cells in the CNS (oligodendrocytes) and the PNS (Schwann cells). <u>Prothymosin</u>, a nuclear protein, and <u>thymosin-alpha1</u>, were found present in <u>astrocytes</u> of normal human brain and could play a role in cell proliferation and gliosis. The IgM of certain patients with <u>paraproteinemic polyneuropathies</u> has been identified as a specific antibody to <u>acidic glycolipids</u>; intraneural injection of IgM in the sciatic nerve of the cat induced demyelination suggesting a direct role in the pathogenesis of the neuropathy. The nature of <u>amyloid protein</u> in patients with "sporadic" <u>amyloid polyneuropathy</u> was identified using specific antibodies to amyloid proteins; <u>point mutations</u> and direct sequencing of <u>prealbumin</u> genes, the precursor protein, were studied in the amyloid tissue using the polymerase chain reaction. The mechanism of inflammatory myopathy in monkeys with <u>immunodeficiency (Simian AIDS)</u> caused by SRV-1 and SIV-1 retroviruses, was studied. Antibodies to SRV-1 immunoreacted with <u>inflammatory cells invading muscle fibers</u>; SRV-1 was capable of infecting <u>myoblasts</u> in tissue culture without exerting a cytopathic effect in the muscle. The role of SIV-1 is similarly studied. The effect of <u>aging on the neuromuscular system</u> of monkeys from age 5 to 25 is being studied with a detailed morphological and morphometrical analysis of their muscle and nerve biopsies. The mechanism of <u>muscle regeneration</u> is studied examining markers on <u>satellite cells</u> including the role of adhesion on molecules such as laminin, N-CAM, and ICAM. The monoclonal antibody Leu-19 (NKH) that identifies natural killer cells was found to share common antigenic determinants with the satellite muscle cells. NKH also stains regenerating muscle fibers and could play a role in <u>muscle regeneration</u>.         </p> <p>*Transferred from IDB on 10/1/88.</p> |   |   |





## ANNUAL REPORT

October 1, 1988 through September 30, 1989

### Neuroepidemiology Branch

Clinical Neurosciences Program, DIR  
National Institute of Neurological Disorders and Stroke

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Annual Report  
October 1, 1988 through September 30, 1989

Neuroepidemiology Branch  
Division of Intramural Research  
Clinical Neurosciences Program  
National Institute of Neurological Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Acting Chief

The Neuroepidemiology Branch (NEB) is responsible for the development and implementation of epidemiologic and genetic programs to investigate the cause, prevention, and treatment of neurologic disorders in human populations. Emphasis has been placed on major neurologic diseases in which the diagnoses can be clinically verified to the satisfaction of skilled neurologists.

On July 14, 1987, Bruce S. Schoenberg, M.D., Dr.P.H., Chief of the Neuroepidemiology Branch since its inception, passed away at a very early age. A permanent Branch Chief is expected to be appointed by January 1990.

Neuroepidemiologic research studies require collaboration of many individuals. However, since there is a severe shortage of available manpower in neuroepidemiology, the Branch has developed an information program for current and future collaborative investigators. A series of six videotapes on aspects of neuroepidemiologic methods produced by the Branch is available. A monograph entitled Neurological Epidemiology: Principles and Clinical Applications was published in 1978. A new monograph which will cover current methodology and disease specific overviews of etiology, incidence, prevalence and intervention studies is in preparation.

Another important problem for the neuroepidemiologist is the enormous cost of maintaining neurologic surveillance on a large number of patients. Therefore, we have attempted to utilize existing registries of neurologic diseases. We collaborate, for example, with the Mayo Clinic in Rochester and utilize their record-linkage system to study neurologic diseases in the population of Rochester, MN; the California Birth Defects Monitoring Program registry; the Israeli National Disease Registry; and the Israeli Cancer Registry.

### Epilepsy

Epilepsy is a major cause of morbidity and mortality on a world-wide basis. A considerable research effort has been devoted by personnel of the Branch to studying this disease.

A protocol is in place for a clinical study of the Lennox-Gastaut syndrome(LGS), a severe childhood epileptic encephalopathy with significant morbidity and mortality, characterized by uncontrolled seizures, mental retardation, and possible mental deterioration. Evaluations include sleep studies, auditory evoked potentials, EEGs and videotape-EEG monitoring, psychologic testing, and a battery of immunologic tests. Neuroimaging studies obtained under an ongoing protocol will be evaluated with the clinical data.

The project is being undertaken in collaboration with the Clinical Epilepsy Section, DIR, NINDS.

In collaboration with the University of Skopje in Yugoslavia, we are examining the utility of the electroencephalogram as a predictor of recurrence of febrile seizures in a defined population in the Region of Macedonia. Data from the Collaborative Perinatal Project was utilized to assess risk factors for febrile seizures, using relative risk and population attributable risk as measures of association in this prospective followup study.

Computerized data are available for all singleton livebirths in military hospitals for a four-year period, 1980 through 1983, a database including 375,310 infants. Three subsequent years of information are ready for inclusion. The occurrence of seizures in the nursery period in these infants, as identified through ICD-9 codes at discharge, and information on maternal and birth factors has formed the basis for a descriptive and analytic study of neonatal seizures. Controls were selected at random from the population of children without seizures. A variety of maternal, pregnancy, birth, and neonatal characteristics are under investigation as predictors of neonatal seizures. Four-hundred thirty infants of the first 275,310 experienced one or more seizures in the nursery period, an overall rate of 0.9 per thousand. Seizure rates were not different by maternal race. Preliminary analysis indicates that seizures occurred twice as often in infants born by cesarean section, an observation consistent with the literature. We are currently examining the factors that might concurrently relate to both the risk for neonatal seizures and the likelihood for cesarean section, that might have produced this observed result. Congenital malformations in the child were approximately three times as common in infants with seizures as in controls.

The international literature indicates that many persons who experience a first convulsion do not have a recurrence in the subsequent two to five years, while many others develop chronic epilepsy. It is unknown whether treatment early after an initial seizure can reduce the likelihood of developing chronic epilepsy. NEB is collaborating with the Biometry and Field Studies Branch in examining the feasibility of randomized controlled trials of treatment with anticonvulsant medication following a first convulsion in subjects ages seven to 60 years who present for care to the Beijing Tiantan Hospital, a municipal hospital affiliated with the Beijing Neurosurgical Institute and to hospitals in the Western Galilee Region of Israel. The initial efforts are focusing on testing the feasibility of recruitment and standardization of procedures and piloting of protocols. The number of patients meeting study criteria, the ease of identification of eligibility, the proportion of patients to be excluded for each indication, the establishment and testing of rules for initiation of treatment in the placebo arm (e.g., would patients continue to accept placebo after a first recurrence), the willingness of patients to participate and return for scheduled visits, the time from seizure onset to study entry, will all be evaluated. Pharmacy procedures in obtaining placebo, and establishing and maintaining the blind will also be tested. Procedures for the informed consent will be developed by staff.

#### Alzheimer's disease

Another neurologic disease of increasing importance in most of the developed countries, including the U.S., is Alzheimer's disease. This is another area

of major research interest to Branch personnel. Uniform diagnostic criteria are applied to all studies of Alzheimer's disease being conducted in the Branch. The clinical diagnosis is based on the criteria proposed by the NINDS- Alzheimer's Disease and Related Disorders Association, Inc. work group. Where possible, pathological verification of the diagnosis is obtained.

Alzheimer's disease/senile dementia, despite its high apparent clinical frequency among the elderly, has not been well studied in a U.S. population. Descriptive studies are yielding etiologic hypotheses which can be further investigated using the case-control approach, and will provide the opportunity to investigate dementia occurring with other neurologic disorders, such as Parkinson's disease. These studies should also provide evidence whether Alzheimer's disease occurring in the very elderly and that in the presenium represent the same disease process. Utilizing a common protocol will permit international comparisons.

In addition, three case-control studies to determine risk factors for Alzheimer's disease have been completed or are in progress. The first uses cases and controls selected from the Rochester, MN population. Past medical records have been utilized to obtain information concerning possible associations between Alzheimer's disease and other medical conditions or surgical procedures. This study has the advantage that recall bias cannot affect the results of the study since data are being abstracted from medical records. Information on the occurrence of head trauma with loss of consciousness (LOC) was abstracted from the medical records of 274 cases and controls. A statistically significant difference in LOC between the two groups was not detected.

A second case-control study of factors associated with Alzheimer's disease in collaboration with the Burke Rehabilitation Center, White Plains, New York, has been completed and analysis is in progress. A third case-control study of possible environmental etiology is underway at Mt. Sinai (New York) Department of Community Medicine and Neurology. The study, using interviews for deriving past environmental exposures, has enrolled approximately 65 patients and an equal number of hospital based controls. Data collection is expected to be completed by the end of FY 1989.

#### Analysis of death certificate data

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed mortality information on some neurologic diseases for the entire U.S. is not available. Analysis of mortality data can be particularly useful for some neurologic diseases because these may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated, or a contributory cause of death to get more complete information about the relationship between the disease and death. Association of diseases occurring at the time of death was studied for all deaths occurring in the U.S. for many neurologic diseases. Diseases occurring together may provide important information in the search for etiology of diseases. Such detailed analysis of mortality data have been done for Huntington's disease, Alzheimer's disease and related diagnoses, Friedreich's disease, motor neuron disease, Parkinson's disease, etc., for



1971-78. The overall patterns which have emerged have been useful in evaluating trends over time and in formulating etiologic hypotheses.

All death certificates for the entire U.S. for the years 1971, and 1973 through 1978 were searched for the diagnosis of Huntington's Disease. Age-, race-, and sex-specific mortality rates for deaths due to and deaths with Huntington's disease were calculated. Time trends in the age-adjusted mortality rates between 1971 and 1978 were also calculated. To determine which conditions may be associated with reduced survival in patients with Huntington's Disease, all death certificates in the United States for 1978 on which Huntington's disease was mentioned have been studied. Each case was compared with two control deaths which were matched for age, race, sex, county and year of death. In the case-control study, pneumonia, choking, nutritional deficiencies, and chronic skin ulcers were increased in cases relative to controls. The overall mortality rate was 2.27 per million population per year.

All cases of Friedreich's disease (FD) diagnosed between 1945 through 1984 among residents of a defined area of northwestern Italy were ascertained (N=58). These patients were followed to death or to December 31, 1984 (whichever came first) to determine the patterns of survival. The 10-, 20-, and 30-year survival rates were respectively 96%, 80%, and 61%, suggesting a better prognosis than previously reported. Survival of FD patients was poorer than expected from the general population. Survival for males was poorer than females even after adjustment for expected survival. Age of onset was not a significant prognostic factor. Survival for patients diagnosed in 1960 or later was better than for those diagnosed before 1960; however, the difference was not statistically significant.

A study of reported international average annual age-adjusted mortality rates for primary tumors of the nervous system was undertaken for the periods 1951-58 and 1967-73. International comparisons of average annual age-adjusted mortality rates for primary tumors of the nervous system showed marked geographical variation for both study periods. For the majority of countries, the mortality rates increased by at least 40% in the intervening 15-year period, while in 20% of the countries the rates increased by over 100%. The percentage increases varied from 12.2 to 345.5. The improvement in the diagnosis of these tumors, particularly among elderly individuals (who have the highest age-specific incidence rates for these neoplasms), presumably accounts for most of this change.

Potential risk factors for various types of stroke were studied using a case-control study design. All the U.S. death certificates in 1978 which had the registered underlying cause of death as subarachnoid hemorrhage (SAH), cerebral hemorrhage (CH), or cerebral infarction (CI) were identified. In a case-control study for potential risk factors for various types of stroke, the data provided new information, such as the occurrence of peripheral vascular disease in association with SAH, the risk of CH in epileptic and cirrhotic patients, and the association of benign neoplasms of the nervous system, motor neuron disease, and 'paralysis agitans' with CI.



## Prevalence surveys

There is continuing controversy regarding racial differences in the occurrence of major neurologic diseases. Thus, a study was conducted in Copiah County, Mississippi to specifically address this question. A strategy was developed which eliminated the requirement that persons must have entered the health care system for detection of disease. The basis of the investigation was a door-to-door survey which utilized a detailed questionnaire inquiring not only about diagnoses, but also about signs and symptoms suggestive of neurologic dysfunction. Over 99% of the households agreed to the interview. Those household members suspected of having one of the disorders of interest were then asked to have a neurologic examination conducted by a senior, board-certified neurologist. This two stage procedure has proven most effective, and the strategy for this type of survey is being utilized in other areas of the world.

Prevalence and incidence data from different parts of the world is often not directly comparable because of the use of different definitions of disease, and results affected by the availability and accessibility to experts in neurology. A uniform protocol developed by the Branch in collaboration with the World Health Organization has helped diminish these differences. The protocol which is being used to estimate the prevalence of major neurologic disorders in many parts of the world, will enable international comparisons.

The prevalence of a wide spectrum of neurologic disorders using the World Health Organization protocol has been reported, based on the Copiah County door-to-door model. Research protocols for these neurologic studies have been completed in India and Spain. The prevalence study of the Parsi Community of Bombay, India provided point prevalence estimates of 328.3/100,000 for Parkinson's disease and 1591.7/100,000 for essential tremor. In the pilot prevalence study in an urban area of Madrid, Spain, migraine and epilepsy were the most frequent disorders with point prevalence ratios of 163.3/1000 and 9.3/1000, respectively.

A large prevalence survey for Amyotrophic Lateral Sclerosis - Parkinsonism Dementia Complex of Guam (ALS-PDC) was undertaken in three rural villages in southern Guam. The survey, utilizing a door-to-door approach, screened all individuals 40 years and over for ALS-PDC. Only rare cases of ALS were encountered, though PDC was found to be still common in the population. Data editing is in progress and comprehensive analysis and publication of results will follow completion of the final data file.

## Neurogenetics

The Clinical Neurogenetics Unit of the Neuroepidemiology Branch seeks the cause, cure and prevention of specific neurologic diseases. For those diseases, a balanced search is made for both genetic and environmental factors. Activities and responsibilities of the unit include clinical care, clinical and laboratory research, teaching, and liaison with several patient organizations.

Over 1000 families, some consisting of over 500 individuals, have been studied in the course of research on single neurogenetic disorders such as the neurofibromatoses, the hereditary leukodystrophies and the hereditary ataxias.

More than 200 twin pairs and their families have been evaluated in the course of study on complex neurological disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.

Clinical care is delivered through the NIH Interinstitutes Genetics Clinic. Presently Dr. Eldridge with a team of 8 specialists participate in the screening, diagnosis, evaluation and management of persons at high risk for selected neurologic disorders, particularly neurofibromatosis 1 (NF1) or von Recklinghausen's disease, and NF2 or bilateral acoustic neurofibromatosis. In the early part of this decade, Dr. Eldridge and Dr. Dino Myrianthopoulos pointed out that the neurofibromatoses together constituted the most common of all neurogenetic conditions. The latter disorder, has recently been shown to be due to a mutation of a single gene in the region of chromosome 22. Subsequently the gene for NF1 has been localized to chromosome 17 confirming that they represent two distinct forms of NF.

Since NF2 is often associated with multiple brain and spinal cord tumors in addition to acoustic neuromas, with over 10 meningiomas, neurofibromas and astrocytomas in some cases, management of an individual case requires careful coordination. Since we now consider NF2 a preventable cause of deafness, facial disfigurement and even death, we have tried to meet the challenge of optimum management.

An approach has evolved involving two other neurosurgical centers, and of over 50 cases followed during the past three years only one individual has died (a man in his 30's whose first tumor was noted at age 18) and only one other has become completely deaf (a 42-year old female with 6 brain tumors and deteriorating neurological status). We are gratified with this record for a condition that generally has been associated with considerable morbidity and premature death.

Clinical research has consisted of: description of the nosology and natural history and inheritance pattern of a number of neurogenetic diseases based on family study; genetic epidemiologic studies seeking either factors influencing the severity of the disorder often using sibs or co-twins as one of the control groups; formal descriptive neuroepidemiologic studies of selected, defined populations; and evaluation of treatment programs using our extensive patient series.

Laboratory studies have been carried out with collaborators in a number of fields including: brain imaging with CT scan, MRI or PET; cell biology looking for factors influencing growth of NF2 tumor cells; molecular genetics for gene mapping or gene isolation and characterization; and neuropathology for detailed anatomic and neurochemical analysis of brain tissue.

Teaching responsibilities begin with students and fellows in the NIH Interinstitutes Genetics Program and the clinical staff at NIH and other area hospitals, and extends to professional and lay groups interested in the combined genetic-epidemiologic approach we employ to understand specific neurologic disease. In the past year presentations have been made at national meetings in New York City, Baltimore, Chicago, plus international meetings in Lisbon, Portugal and Riyadh, Kingdom of Saudi Arabia.

An especially active and successful area of responsibility for the Neurogenetics Unit involves liaison with a number of voluntary lay organizations. Over the years Dr. Eldridge has assisted in the formation of eight local or national foundations, including the National Neurofibromatosis Foundation and the Washington, D.C. chapter; the Dystonia Medical Research Foundation and the Washington, D.C. chapter; the National Jewish Genetic Disease Foundation, the Washington, D.C. chapter of the National Ataxia Foundation, Neurofibromatosis, Inc. and its local chapter; and most recently iNFormer 2 which is a network for NF2 families. We have tried to help these organizations with advice. They in turn have enabled us to perform three recent clinical studies by enlisting the cooperation of their membership.

Recent work of the Neurogenetics Unit include the following for single gene disorders:

NF2 - The natural history of NF2 appears different in males with the gene than it does in females. Affected men tend to have fewer tumors, require less surgical intervention and, when they pass on the gene, have it expressed later in their children than their affected sisters do. A pathognomonic sign has been described by our team. It is the presence at a young age of one or more discreet opacities in the posterior portion of the lens of the eye which can appear before any other signs or symptoms. With hand-held ophthalmoscope it is seen as a black fleck and is known locally as the 'Kaiser-Kupfer Cinder'. A review has been prepared on the practical management of NF2, which makes the following novel points: delineates 5 groups at high risk for NF2; emphasizes that no one should be operated on for a presumed solitary, sporadic acoustic neuroma unless this syndrome with bilateral acoustic neuromas is first ruled out; and that watchful waiting rather than aggressive surgical intervention is the proper course in the majority of cases.

NF1 - We have published a rigorous study of cognitive and neuropsychological function in NF1. A study is ongoing of an appraisal of attitudes of over 300 affected individuals and their relatives toward NF1 itself and toward predictive testing for NF1.

Hereditary Leukodystrophy - We have described an adult form which has been often misdiagnosed as chronic progressive multiple sclerosis. Four families in the U.S. have now been ascertained. A high concentration of a previously undescribed familial leukodystrophy of early onset has been identified by colleagues in Saudi Arabia.

Among the complex, or multifactorial, neurologic disorders, recent findings include:

Alzheimer's disease - demonstration in the first and only nationwide twin study that most MZ twins are discordant and remain so for over 12 years, indicating that environmental factors are important in many cases.

Multiple sclerosis - We have noted the similarity between MS and paralytic polio concordance rates in MZ and DZ twins, indicating that environmental and genetic factors both must play a role in each condition.



Parkinson's disease - We have demonstrated in the first nationwide twin study in the U.S. extraordinarily low concordance rates, a finding since confirmed in English and Finnish studies. In the U.S. study there were far more U.S. full sibs and parents affected than co-twins, suggesting a familial tendency to Parkinson's and protective effect on the co-twin. A characteristic life-long personality difference suggested an early environmental event 'set' a twin's risk. These observations plus mouse data indicating genetic influence on dopamine neuron number led to the 'Initial Neuron Number', or INN, theory as one of the processes contributing to Parkinson's disease. If true it would mean that there is a humoral factor capable of reducing one's risk- or of treating one's symptoms.

A future goal of the Unit is to see the convening of a consensus conference sponsored by NINDS, NCI, NIDCD, & NEI dealing with acoustic neuroma. This is a brain tumor, which when bilateral, is the hallmark of NF2. It is often not well managed in the young population but information and technical developments exist to prevent the all-too-common consequences of the tumors -- deafness, facial disfigurement and diminished vision.

While we have programs underway or contemplated in all the areas mentioned above, there are over a thousand single gene disorders, and we are addressing less than 10. There are several million individuals with Alzheimer's disease, multiple sclerosis, or Parkinson's disease. We have, with limited resources, made fundamental observations regarding each of these conditions based primarily on a unique population of twins, and suggested etiological studies for each.

#### Pediatric neuroepidemiology

In the area of pediatric neuroepidemiology, the efficacy and side effects of phenobarbital in young children with febrile seizures, randomized to phenobarbital or placebo is being studied. A number of endpoints have been or will be assessed: IQ, seizure recurrence, sleep pattern, temperament scale, and a number of behavioral and cognitive measures. The design of this study permits comparison of measures of tested intelligence and of behavior in children with febrile seizures who were treated with phenobarbital, in those randomized to placebo, and in a group of seizure-free control children. It was presumed that the group randomized to placebo would experience more recurrences of seizures, while the group assigned to phenobarbital might be subject to unfavorable side effects of medication. This comparison allows assessment of benefit and risk of treatment in a common childhood neurologic problem. Children in this study were also tested six months after completion of receipt of phenobarbital, so that the reversibility of any observed effects could be judged. All subjects have been recruited, followup testing has been completed, and the initial manuscript describing the primary results has been submitted for publication. Work has begun on other manuscripts from this data.

Studies of dental and dermatoglyphic markers of maldevelopment have been initiated in cooperation with the National Institute of Dental Research and the Institute of Aging. These studies of enamel defects and characteristics of dermatoglyphics and palmar creases in children with cerebral palsy or mental retardation will attempt to recognize the occurrence and timing of events adverse to neurologic development.

NEB staff were involved in the analysis of a randomized controlled clinical trial undertaken in the NICHD to evaluate the safety and efficacy of phototherapy in the prevention/reduction of hyperbilirubinemia in the neonate. An earlier study indicated the efficacy of the treatment in preventing hyperbilirubinemia. This phase investigated the frequency of adverse outcomes, most of them neurological, in this sample at follow-up examination at six years of age. Infants randomized to treatment with phototherapy experienced lower ranges of bilirubin as neonates but did not experience increased frequency of adverse neurologic outcomes.

NEB staff in collaboration with the California Birth Defects Monitoring Program, and the Health Officers Association of California, is establishing a population-based registry of children with cerebral palsy (CP) in five counties of the San Francisco Bay Area born between 1983-1985. Children are ascertained through client records at California Children Services and the Department of Development Services and their Regional Centers for the Developmentally Disabled. CP cases meeting the registry criteria will receive a clinical examination to establish a research diagnosis, and data will be obtained on associated features. Obstetric and pediatric records will be reviewed by the staff. Information will be used to establish trends in incidence, to examine for time-space clustering, and to formulate hypotheses for testing suspected etiologic factors, medical and environmental, in case-control studies. Ecological studies will also be conducted comparing rates of CP among births in Santa Clara County census tracts which contain EPA superfund waste disposal sites, to rates in the county as a whole and to rates in the remainder of the Bay area; the purpose of this aspect of the project is to examine the possibility that toxic exposures may contribute to some forms of neurologic morbidity as well as to other birth defects. Examinations for case classification are in progress. It is anticipated that 300 cases will be examined by the end of calendar year 1989.

#### Rare disorders of the nervous system

Emphasis is placed on research on the major diseases of the nervous system, and the Branch also investigates other diseases which may be less frequent or less debilitating, but important in terms of pathogenesis or clues to disease etiology.

In collaboration with other governmental agencies (Centers for Disease Control), other governments (Colombia, Republic of Seychelles), international organizations (World Health Organization), and universities (Mayo Medical School), methods have been developed to investigate less common neurologic disorders. Several such studies have been completed as: a prevalence and case-control study of progressive supranuclear palsy (PSP); descriptive and analytic studies of spastic paraparesis in different parts of the world; and a door-to-door survey of 21 provinces of the People's Republic of China to determine the prevalence of migraine.

The prevalence and natural history survey of PSP in two counties in New Jersey provided a prevalence ratio of 1.39/100,000. The median intervals to onset of requiring assistance was 3.1 years; of visual symptoms, 3.9 years; dysarthria, 3.4 years; dysphasia, 4.4 years; requiring wheel chair, 8.2 years; and death 9.7 years. In the case-control study of PSP with two matched controls, as adults, PSP cases lived in areas with low population significantly more



frequently than controls. The study identified no other factors associated with PSP including a history of stroke, hypertension, or smoking. The prevalence survey of migraine in the People's Republic of China yielded a point prevalence ratio of 690/100,000. The prevalence ratio of migraine for females was higher than that for males.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01924-19 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, Pathophysiologic Study of Hereditary Movement Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                              |                    |                 |
|------------------------------|--------------------|-----------------|
| P.I.: Roswell Eldridge, M.D. | Medical Geneticist | NEB, DIR, NINDS |
| Others: Robert Cohen, M.D.   | Chief              | LBI, IRP, NIMH  |
| Elizabeth Mathew, M.D.       | Neurologist        | LBI, IRP, NIMH  |

## COOPERATING UNITS (if any)

CNB, DIR, NINDS; LCS, DCBR, NIMH; Dept. of Neurology, University of Mississippi School of Medicine

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.35

## PROFESSIONAL:

0.3

## OTHER:

0.05

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In this project, we seek to 1) clarify and expand the nosology of the hereditary movement disorders; 2) contribute to the understanding of the underlying biochemical basis; 3) determine the most effective treatment and predictive testing for each disorder; and 4) suggest guidelines for counseling individuals at risk. General syndromes under study include the dystonias, tic disorders, the ataxias and myoclonus. Approaches include standard epidemiologic and clinical genetic studies, and collaborative efforts in evaluating the role of neurotransmitters such as dopamine, and PET Studies.

Collaborative studies are underway to explain our earlier observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and bipterin in CSF in a genetic subset of dystonia patients. Members of selected families are admitted to the Clinical Center, NIH, for trials of several new pharmacological agents. Biopterin administered intravenously has led to acute benefit in one form of generalized dystonia.

We are performing PET studies of at risk members of three large kindreds with hereditary ataxia. Preliminary results suggest: a more generalized involvement than previously recognized; presymptomatic changes by PET; and evidence for genetic heterogeneity.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01927-19 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                            |                      |                 |
|---------|----------------------------|----------------------|-----------------|
| P.I.:   | Roswell Eldridge, M.D.     | Medical Geneticist   | NEB, DIR, NINDS |
| Others: | Bracie Watson, M.S.        | Geneticist           | NEB, DIR, NINDS |
|         | Murial Kaiser-Kupfer, M.D. | Geneticist           | OGB, IP, NEI    |
|         | Anita Pikus                | Audiologist          | CA, IP, NIDCD   |
|         | Wesley McBride, M.D.       | Molecular Geneticist | LBC, IP, NCI    |
|         | Dylis Parry, Ph.D.         | Geneticist           | CEB, IP, NCI    |
|         | Donald Sclar, Ph.D.        | Audiologist          | CA, IP, NIDCD   |

## COOPERATING UNITS (if any)

OP, CC: SN, DIR, NINDS; Division of Medical Genetics, Dept. of Pediatrics, Children's Hospital National Medical Center; Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA; Uniformed Services University of Health Sciences

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.2

## OTHER:

0.3

## CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In this project we seek to define and classify hereditary tumors of the nervous system such as occur in neurofibromatosis; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment; and to develop methods for preclinical detection and screening.

Our studies have led to the recognition of a preventable cause of deafness, visual loss or even death: Neurofibromatosis 2 or bilateral acoustic neurofibromatosis. The genes for two distinct forms of neurofibromatosis have now been mapped to specific chromosomes: 1) the classical form as described by von Recklinghausen (neurofibromatosis 1) on #17, and 2) a form in which bilateral acoustic neuromas are the hallmark (neurofibromatosis 2) on #22. Efforts in the latter have been directed at improving and simplifying screening of high-risk individuals, confirming diagnosis, establishing criteria for intervention, and isolating and characterizing the gene. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are useful means for early documentation and monitoring of acoustic neuromas. The role of vestibular studies is being evaluated. Presenile lens opacities or cataracts occur in most cases and may be the initial pathognomonic sign.

Our first major study involving neurofibromatosis, a multi-disciplinary project, demonstrated mild but consistent impairment of neurologic and cognitive status in these patients compared to their unaffected sibs. A study assessing the burden of NFL and attitudes towards predictive testing is nearing completion.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02167-15 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Epidemiology Studies in MS and Other Multifactorial Neurologic Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                          |                                |                 |
|---------|--------------------------|--------------------------------|-----------------|
| P.I.:   | Roswell Eldridge, M.D.   | Medical Geneticist             | NEB, DIR, NINDS |
| Others: | Henry F. McFarland, M.D. | Assistant Chief                | NI, DIR, NINDS  |
|         | Ronald J Polinsky, M.D.  | Neurologist                    | CNB, DIR, NINDS |
|         | Walter A. Rocca, M.D.    | Consultant Neuroepidemiologist | NEB, DIR, NINDS |

## COOPERATING UNITS (if any)

BFSB, NEB, NI, CNB, NINDS; M; Department of Neurology, Monmouth Medical Center, Monmouth, NJ; Italian Multicenter Study on Dementia, S.M.I.D. Centers, Florence, Italy.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we are coupling genetic and environmental studies in selected families, twin pairs & populations with disorders such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease, in an effort to distinguish specific contributing factors.

A multi-disciplinary twin study of Parkinson's disease has led to formulation of an etiologic theory we term the "initial neuron number" hypothesis. Since neurons in the substantia nigra are not known to regenerate but rather appear to die off at a constant rate during adulthood, starting life with a reduced number of these critical neurons may be one predisposing factor to eventual development of the disorder.

A study similar in design involving twins with dementia of the Alzheimer's type also indicates environmental factors must be involved in some forms of the disorder.

An autosomal dominant, hereditary leukoencephalopathy simulating MS with onset at about age 35 is under study in a kindred with over 20 affected. Derangement of the autonomic nervous system is often seen early in the course and when recognized clinically, serves to distinguish this single gene disorder from multiple sclerosis. Computerized tomographic scan changes of the brain are characteristic, even in early cases.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02240-13 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiology of Dementia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Jonas H. Ellenberg, Ph.D. Acting Chief NEB, DIR, NINDS

## COOPERATING UNITS (if any)

Vijay Chandra, M.D., Ph.D. New Dehli, India; Francis M. Baker, M.D., University of Texas Health Science Center.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analytic studies to determine risk factors for Alzheimer's disease (AD) are planned or being conducted. A large case-control study of head trauma with loss of consciousness as a risk factor for AD has been completed in Rochester, MN. There was no detected increase in risk of AD following head trauma. A case-control study of Alzheimer's disease is in progress in White Plains, New York. An attempt has been made to evaluate and improve instruments used in case-control studies of dementia.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02243-13 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pediatric Neuroepidemiology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                           |                 |                  |
|---------|---------------------------|-----------------|------------------|
| P.I.:   | Karin B. Nelson, M.D.     | Medical Officer | NEB, DIR, NINDS  |
| Others: | Sherrie Emoto, Ph.D.      | Staff Fellow    | BFSB, DIR, NINDS |
|         | Jonas H. Ellenberg, Ph.D. | Acting Chief    | NEB, DIR, NINDS  |

## COOPERATING UNITS (if any)

J.F. Mellinger, M.D., M.R. Gomez, M.D., L.T. Kurland, M.D., M. Cruz, M.D., and R.V. Groover, M.D., Dept. of Neurology, Mayo Clinic; Cheryl Naulty, M.D., Walter Reed Hospital; Peter Scheidt, M.D., NICHD; Judith Grether, Ph.D., Dept. of Health Services, California.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.4

## PROFESSIONAL:

0.3

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Several studies in pediatric neuroepidemiology are ongoing: 1) analysis of case-control data for putative predictors of febrile seizures, based on a data set from six cities from the People's Republic of China has been completed; 2) the California Birth Defects Monitoring Program, in conjunction with the Health Officers Association of California, is establishing a population-based registry of children with cerebral palsy (CP) in five San Francisco Bay Area counties, for monitoring of trends in incidence and for the creation of a case-control registry for studies of suspected etiologic factors, medical and environmental; 3) NINDS participants were involved in the analysis phase of a randomized controlled clinical trial undertaken in the NICHD to evaluate the safety and efficacy of phototherapy in the prevention/reduction of hyperbilirubinemia in the neonate. We investigated the frequency of adverse outcomes, most of them neurological, in this sample at followup examination at six years of age.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02297-13 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mortality from Neurologic Disorders: National and International Comparisons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Jonas H. Ellenberg, Ph.D. Acting Chief NEB, DIR, NINDS

## COOPERATING UNITS (if any)

E.W. Massey M.D., Duke University; W.A. Rocca M.D., Firenze, Italy; N. Mantel, American University

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

.05

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed mortality information on some neurologic diseases for the entire U.S. is not available. Analysis of mortality data can be particularly useful for some neurologic diseases because these may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated, or contributory cause of death to get more complete information about the relationship between the disease and death. Association of diseases occurring at the time of death are also being studied for all deaths occurring in the U.S. for many neurologic diseases. Diseases occurring together may provide important information in the search for etiology of diseases.

Detailed analyses of mortality data have been accomplished for Huntington's disease (comorbidity at time of death), Friedreich's disease (survival analysis), international comparisons of average annual age-adjusted mortality rates for primary tumors of the nervous system, and stroke (case-control study of risk factors).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02299-13 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reviews of Epidemiologic Aspects of Neurologic Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Jonas H. Ellenberg, Ph.D. Acting Chief NEB, DIR, NINDS

## COOPERATING UNITS (if any)

N. Bharucha, M.D., Bombay, India; William Koller, M.D., Ph.D., University of Kansas; Ian Irwin, Ph.D., Institute for Medical Research, San Jose.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been given to: cerebrovascular disease; otitis media; inherited ataxias; Huntington's disease; febrile seizures; Tourette's syndrome; peripheral neuropathy; neurologic disease in the elderly; controlled therapeutic trials of motor neuron disease; epilepsy; descriptive, analytic, and experimental methods in neuroepidemiology; procedures for neuroepidemiologic investigations in developing countries; and epidemiologic studies of Alzheimer's disease; myasthenia gravis; cerebral malaria; and autism.

|   |                      |                                       |
|---|----------------------|---------------------------------------|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                      | PROJECT NUMBER<br>Z01 NS 02300-13 NEB |
| PERIOD COVERED<br>October 1, 1988 through September 30, 1989  |                      |                                       |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)<br>Clinical Course and Medical Care for Neurologic Disorders  |                      |                                       |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>Acting P.I.: Jonas H. Ellenberg, Ph.D.    Acting Chief    NEB, DIR, NINDS  |                      |                                       |
| COOPERATING UNITS (if any)<br>J. P. Whisnant, M.D., Department of Neurology, Mayo Clinic, Rochester, MN; Vijay Chandra, M.D., Ph.D., New Delhi, India; Adesola Ogunniyi, M.D., Nigeria; S. Ouyang, M.D., Changsha, China; K. Wang, M.D., Changsha, China.   |                      |                                       |
| LAB/BRANCH<br>Neuroepidemiology Branch, Division of Intramural Research   |                      |                                       |
| SECTION   |                      |                                       |
| INSTITUTE AND LOCATION<br>NINDS, NIH, Bethesda, Maryland 20892  |                      |                                       |
| TOTAL MAN-YEARS:<br>0.0   | PROFESSIONAL:<br>0.0 | OTHER:<br>0.0                         |
| CHECK APPROPRIATE BOX(ES)<br><input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither<br><input type="checkbox"/> (a1) Minors<br><input type="checkbox"/> (a2) Interviews   |                      |                                       |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)<br><br>The study uses a review and abstraction of data from records for a selected group of <u>neurological disorders</u> . It obtains the items of data necessary to determine onset of the disorder, comorbid conditions, duration, date and cause of death, or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve, and morbidity and severity estimates. It will also include analysis of type and duration of medical care received by patients with neurologic disorders derived from a well-defined population. |                      |                                       |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02301-13 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Collaborative Studies of Less Common or Less Debilitating Neurologic Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Jonas H. Ellenberg, Ph.D. Acting Chief NEB, DIR, NINDS

## COOPERATING UNITS (if any)

Zhao Fu, M.D., Beijing, China; P. Davis, M.D., Shreveport, LA; G. Roman, M.D., Lubbock, Texas; L. Golbe, M.D., New Brunswick, New Jersey.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of collaborative efforts involve the investigation of the characteristics of unusual or less debilitating (e.g., headache) neurologic disease phenomena. Unusual associations or space/time clusters of neurologic disorders may provide leads to etiology or therapy. These may be tested through more formal approaches.

Surveys of prevalence and natural history and a case control study of progressive supranuclear palsy (PSP) have been completed in two counties in New Jersey. The point prevalence was 1.39/100,000. The median intervals to onset of requiring assistance was 3.1 years and to death was 9.7 years. The case control study did not implicate a history of stroke, hypertension or smoking as risk factors for PSP. A prevalence survey of migraine in the People's Republic of China yielded a point prevalence ratio of 690/100,000, and indicated that the prevalence ratio for females was higher than that for males. Surveys for Parkinson's disease are being conducted in former Far East prisoners of war.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02307-13 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Educational Resources in Neurological Epidemiology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Jonas H. Ellenberg, Ph.D. Acting Chief

NEB, DIR, NINDS

Other: Dallas W. Anderson, Ph.D. Mathematical Statistician BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because there is severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six video tapes produced by the Branch are distributed on a loan basis without charge. A monograph, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS was published in 1978. A new monograph which will cover current methodology and disease specific overviews of etiology, incidence, prevalence and intervention studies is in preparation.

A set of video tapes have been produced for training interviewers in the methodology of interviewing for case-control studies. This has been done in both Italian and in English.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02370-11 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Racial and Geographic Differences in Occurrence of Neurologic Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Jonas H. Ellenberg, Ph.D. Acting Chief NEB, DIR, NINDS  
Other: Dallas Anderson, Ph.D. Mathematical Statistician BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

OBFS, OD, NINDS: A. Haerer, M.D., Univ. of Mississippi; U.S. Bureau of the Census; N. Bharucha, M.D. (India); M.C. Gutierrez del Olmo, M.D., A. Portera-Sanchez, M.D. (Spain).

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The purpose of these studies is to accurately document possible racial, environmental and geographic differentials in the prevalence of major neurologic disorders by surveying an entire geographically defined population. The disorders investigated include cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, multiple sclerosis and cerebrovascular disease.

Research protocols for these neurologic studies have been completed in the United States (Copiah County), India and Spain. The prevalence study of the Parsi Community of Bombay, India provided point prevalence estimates of 328.3/100,000 for Parkinson's disease and 1591.7/100,000 for essential tremor. In the pilot prevalence study in an urban area of Madrid, Spain, migraine and epilepsy were the most frequent disorders with point prevalence ratios of 163.3/1000 and 9.3/1000, respectively.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02423-10 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Data Resources for Neuroepidemiology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Acting P.I.: Lawrence Lavine, D.O., M.P.H. Medical Officer NEB, DIR, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.00

## PROFESSIONAL:

0.00

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To develop 1) a registry of hospitalized patients with neurologic disease in a geographically well-defined population; 2) resources for case-control studies of neurologic diseases using uniform methods of data collection; and 3) a registry of neurologic disease in the well-defined population of the United States military.

This project is temporarily on hold pending the appointment of a permanent Branch Chief and appropriate staff recruitment. The feasibility of continuation of this project will be assessed at that time.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02570-07 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Natural History of ALS-PD in Guam

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Lawrence Lavine, D.O., M.P.H. Medical Officer NEB, DIR, NINDS  
Other: Zhen-xin Zhang, M.D. Guest Researcher NEB, DIR, NINDS

## COOPERATING UNITS (if any)

Leonard Kurland, M.D., Mayo Clinic, Rochester, MN; John Steele, M.D., Veterans Administration, Guam; Xhen-xin Zhang, M.D., People's Republic of China; Donald Calne, M.D., Univ. of British Columbia, Vancouver, BC; Peter Spencer, Ph.D., Albert Einstein College of Medicine, NY.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As a continuation of previous projects on clinical, pathological, and epidemiologic surveillance of Guamanian amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia (PD) in the Marianas Islands, identified survivors, including suspects, in the NINDS Registry as of January 1, 1983, were followed at intervals of six months for detailed clinical descriptions of patterns of progression, by a qualified neurologist. The patients still alive have entered a chronic state and final exams were completed in the Fall of 1988. As these patients expire, attempts will be made to obtain the appropriate pathologic material for examination by various neuropathologists around the world. All attempts to examine this material should be made because of the uniqueness of these patients.

The prevalence survey of three southern Guamanian villages to determine the current prevalence of ALS/PDC was completed in 1988. As this is a transitional society, dietarily and culturally since WW-II, a simultaneous survey for other major neurologic diseases and major diseases of aging populations was also performed.

A chart review study using the NINDS Guam registry for ALS/PDC for the period 1944-85 was performed. Downward trends in age-adjusted incidence rates and upward trends in age of onset were seen for both sexes. Since 1980, new cases occurred only among persons over 50 years of age, whereas younger age at onset had been noted in the past.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02715-04 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Epilepsy Neuroepidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|         |                               |                  |                      |
|---------|-------------------------------|------------------|----------------------|
| P.I.:   | Karin B. Nelson, M.D.         | Medical Officer  | NEB, DIR, NINDS      |
| Others: | Lawrence Lavine, D.O., M.P.H. | Medical Officer  | NEB, DIR, NINDS      |
|         | P. Satish Chandra, M.D.       | Guest Researcher | NEB, DIR, NINDS      |
|         | Jonas H. Ellenberg, Ph.D.     | Chief            | BFSB NEB, DIR, NINDS |
|         | James Dambrosia, Ph.D.        | Statistician     | BFSB, DIR, NINDS     |
|         | William Theodore, M.D.        | Medical Officer  | MNB, DIR, NINDS      |

## COOPERATING UNITS (if any)

P Satischandra, M.D., NIMHANS, Bangalore, India; Shi-chuo Li, M.D., Beijing Neurosurgical Institute, PRC; Judith Manelis, M.D., Western Galilee Regional Hospital, Israel; Gary Eglinton, M.D., Georgetown University School of Medicine.

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

## PROFESSIONAL:

## OTHER:

35

0.3

0.05

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several studies on convulsive disorders are being planned and tested for feasibility, or in progress. A protocol is in effect for a clinical study of the Lennox-Gastaut syndrome (LGS), a severe childhood epileptic encephalopathy with significant morbidity, characterized by uncontrolled seizures, mental retardation, and possible mental deterioration, to define the pathophysiology and anatomic locus of disturbance in LGS. A descriptive and analytic study of neonatal seizures in a large military database is underway. Data are available for all singleton livebirths in military hospitals for a four-year period, a database including 375,310 infants. The occurrence of seizures in the nursery period in these infants and information on maternal and birth factors forms the basis for the study of these characteristics as predictors of neonatal seizures. We are evaluating the feasibility of performing randomized and placebo controlled clinical trials of treatment after an initial convulsion in subjects presenting for care to the Beijing Tiantan Hospital and to a consortium of hospitals in Jerusalem. With Yugoslav colleagues, we are examining the utility of the electroencephalogram as a predictor of recurrence of febrile seizures in a defined population in Yugoslavia.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02746-03 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenobarbital Clinical Trial in Children with Febrile Seizures\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS  
Others: Deborah Hirtz, M.D. Pediatric Neurologist DNB, DCDND, NINDS  
Young Jack Lee, Ph.D. Mathematical Statistician NEB, DIR, NINDS  
Jonas H. Ellenberg, Ph.D. Chief BFSB, DIR, NINDS

## COOPERATING UNITS (if any)

Jacqueline Farewell, M.D., Dept. of Neurosurgery, Univ. of Washington, Seattle

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of the study are: To assess the effects on tests of intelligence and behavior of phenobarbital, a commonly prescribed anticonvulsant in children.

The design of this study permits comparison of measures of tested intelligence and of behavior in children with febrile seizures who have been treated with phenobarbital, and in a group of seizure free control children. A comparison of the groups allows assessment of benefit and risk of treatment for a common childhood neurologic problem.

The continued analysis efforts for the Phenobarbital Clinical Trial will be subsumed under the Intramural Research Project "Epilepsy Neuroepidemiology" (Z01 NS 02715-04). The data collection phase is considered completed.

\*[This study supports the DNB/DCDND/NINDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINDS, and the contractor of the study is the University of Washington.]

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02747-03 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dental Markers of Maldevelopment

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS

## COOPERATING UNITS (if any)

Mohandas Bhat, D.D.S., D.P.H., EODPP, NIDR

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is an exploratory effort to examine, in a group of children with chronic motor disability of early onset and nonprogressive course (cerebral palsy, CP), potential markers of maldevelopment. It will focus on the frequency and nature of dental abnormalities in affected children, in comparison with the frequency of similar characteristics among healthy children.

The objectives are: To examine whether dental abnormalities, especially enamel defects, can serve as markers of maldevelopment, and whether such findings can provide information concerning timing of adverse events or exposures.

The significance of the research is that: Enamel hypoplasias and other dental anomalies can offer clues as to the timing of insults or exposures that occur from the fourth month of gestation to the age of about 12 months of extrauterine life. Correlation of dental with clinical data may offer a means to explore the timing of departure from the normal course of development in a group of children with chronic motor disability of early onset and nonprogressive course (cerebral palsy, or mental retardation).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02748-03 NEB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dermatoglyphic Markers of Maldevelopment

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS

## COOPERATING UNITS (if any)

Chris Plato, Ph.D., GRC, NIA; Mrs. Cathy Fox, University of Maryland

## LAB/BRANCH

Neuroepidemiology Branch, Division of Intramural Research

## SECTION

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fingerprints are in place by nine to 16 weeks of intrauterine life, dermal creases by 19 weeks. The objective of this pilot study is to examine whether dermatoglyphic abnormalities and dermal creases can serve as markers of maldevelopment, and whether such findings can provide information concerning timing of adverse events or exposures in children with abnormal neurologic development. This is an exploratory effort to examine, in a group of persons with chronic motor disability of early onset and nonprogressive course (cerebral palsy, CP), or with mental retardation, potential markers of maldevelopment. It will focus on the frequency and nature of dermatoglyphic abnormalities in affected individuals, in comparison with the frequency of similar characteristics among healthy persons.

The significance of the research is that a time course of establishment of dermatoglyphics and palmar creases has been developed. The hypothesis has been offered, and some data accrued to indicate, that dermal findings may offer clues as to the timing of insults or exposures during early prenatal life, providing a means to explore the timing of departure from the normal course of development in a group of neurologically handicapped children.

Publications:

Eldridge R. Hereditary disorders of the basal ganglia. In: Emery AEH, Rimoin D, eds. Principles and practice of medical genetics. New York: Churchill Livingstone 1989; (in press).

Z0 NS 01927-19 NEB

Eldridge R. Neurofibromatosis 2 (Bilateral acoustic neurofibromatosis). In: Rubenstein A, ed. Neurofibromatosis: a layman's guide. New York: Raven 1988; (in press).

Eldridge R, Denckla MB, Bien E, Myers S, Kaiser MI, Pikus A, Schlesinger SL, Parry DM, Dambrosia JM, Zasloff MA, Mulvihill JJ. Neurofibromatosis: Type 1 (Recklinghausen's Disease) neurological and cognitive assessment with sibling controls. Am J Dis Child 1989;143:833-7.

Kaiser-Kupfer MI, Freidlin V, Datiles MB, Edwards, PA, Sherman, JL, Parry D, McCain, LM, Eldridge R. The association of posterior capsular opacities with bilateral acoustic neuromas in patients with Neurofibromatosis 2. Arch Ophthalmol 1989;107:541-4.

Z0 NS 02167-15 NEB

Eldridge R, Rocca WA. Parkinson's disease: etiologic considerations. In: King RA, Roffer JI, Mofulsky AG, eds. The genetic basis of common diseases. New York: McGraw-Hill; (in press).

Schwankhaus JD, Patronas N, Dorward R, Eldridge R, Schlesinger S, McFarland H. Computed tomography and magnetic resonance imaging in adult leukodystrophy. Arch Neurol 1988; 45:1004-8.

Z0 NS 02240-13 NEB

Amaducci L, Schoenberg BS. Analytical epidemiology and risk factors in Alzheimer's Disease. In: Peock K, Freund HJ, Ganshirt H, eds. Neurology: Proceedings of the XIII World Congress of Neurology. Berlin: Springer-Verlag; (in press).

Chandra V, Kokmen E, Schoenberg BS, Beard CM. Head trauma with loss of consciousness as a risk factor for Alzheimer's disease. Neurology; (in press).

Chandra V, Schoenberg BS. Inheritance of Alzheimer's disease: epidemiologic evidence. *Neuroepidemiology* 1989;8:165-74.

ZO NS 02243-13 NEB

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ANNUAL REPORT

October 1, 1988 through September 30, 1989

Clinical Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

Neuroimmunology Branch

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Annual Report  
October 1, 1988 to September 30, 1989  
Clinical Neurosciences Program, DIR  
National Institute of Neurological and  
Communicative Disorders and Stroke

The Neuroimmunology Branch (NIB) is divided administratively into four groups, the Office of the Chief (OC), the Neurological Diseases Section (NDS), the Molecular Immunology Section (MIS), and the Cellular Immunology Section (CIS). Research includes both fundamental studies of immune function and clinical investigations in patients with neurological diseases. During FY 1989, focus was placed on multiple sclerosis (MS) and a chronic-progressive myelopathy associated with human T lymphocytotropic virus I (HTLV-I). In the past, this disorder has been known by a number of names, but during the past year, agreement of the designation, HTLV-I-associated myelopathy/tropical spastic paraplegia (HAM/TSP) was reached at an international workshop.

A major clinical project in the NIB over the past four years has been a therapeutic trial of cyclosporine A for patients with progressive MS. This was conducted in collaboration with 11 other medical centers. During FY 1989, analyses of the data were completed and showed that cyclosporine A produced small but definite slowing in the rate of progression; however, many patients experienced nephrotoxicity. This side effect potentially prevents the use of this drug in a routine manner. Consequently, a small open-label trial has been initiated. Focus is being placed on defining the degree of renal toxicity and identifying patients prone to develop this side effect. A parallel approach will be to examine the use of agents such as an antagonist of thromboxane to reduce nephrotoxicity associated with cyclosporine A. The overall goal of these approaches is to identify patients and therapeutic regimens that will enable the long-term treatment of patients with cyclosporine A in order to capitalize on the encouraging preliminary results.

The NIB continues to have a major interest in neuroimaging techniques for the identification of lesions in patients with MS and for longitudinal assessment during the clinical course. Within recent months it has become apparent that, during acute exacerbations, T1 weighted magnetic resonance imaging, after the administration of gadolinium, shows enhancing lesions which indicate breakdown in the blood-brain barrier (BBB). These abnormalities could provide clues about the underlying pathogenic mechanisms in the disorder and have potential for assessing the efficacy of therapeutic agents. In order to define the natural history and prevalence of such lesions, a new protocol has been initiated. In this study a small group of active, clinic patients are being evaluated clinically and by gadolinium scanning monthly.

The clinical investigations are closely related to fundamental studies throughout the Branch. For example, experimental allergic encephalomyelitis (EAE) is being extensively studied in the NDS. A goal of these investigations is to identify mechanisms which are involved in the production of immunologically mediated disease in the central nervous system (CNS). Interaction between the immune system and cellular components in the nervous system which form the BBB is believed to be an early event. During FY 1989, a 12 amino acid polypeptide that is encephalitogenic for SJL mice was

identified. T-cell lines that recognize this epitope proliferate in vitro when cultured with the 12 amino acid peptide. In addition, these T-cell lines lyse macrophages and presumably other antigen-presenting cells (APC) that have been pulsed with the encephalitogenic peptide. It was shown previously that under certain experimental situations brain capillary endothelial cells which form the BBB can function as APCs. The in vivo lysis of brain capillary endothelial cells, by cytotoxic T lymphocytes (CTL) with specificity for the encephalitogenic peptides would provide one mechanism for BBB breakdown.

Extensive investigations of CTL possibly related to the pathogenesis of MS are ongoing in the CIS. A new project has been initiated to characterize the CTL response to myelin basic protein (MBP) in MS patients and to compare this with the response in normals. The fine specificity of MBP-specific CTL and the components of the major histocompatibility complex (MHC) that are corecognized by these CTL are being examined. CD4<sup>+</sup> MBP-specific CTL have been obtained from the blood and CSF from patients with MS. Detailed studies in a few patients indicate that the T-cell response is relatively heterogeneous in terms of epitope specificity and T cell receptor (TcR) usage. In addition, MHC molecules involved in antigen presentation vary among different patients.

A second major study in the CIS involves the assessment of CTL directed at the measles virus in MS. Previously, the CIS discovered that measles virus-specific CTL are reduced in most patients with MS. This remains the only antigen-specific abnormality in the disease, and provides rationale for extensive investigation of virus-specific CTL. During FY 1989, these investigations were extended to assessment of CTL in patients with acute measles infection, subacute sclerosing panencephalitis (SSPE), HAM/TSP, and other neurological disorders. Reduced measles virus-specific CTL have been demonstrated in the blood of some patients with SSPE and in patients with HAM/TSP. In SSPE, the abnormality may reflect "homing" of CTL to the CNS. In HAM/TSP, reduced generation of CTL to other viruses such as influenza and mumps, has been found. This is believed to indicate a generalized defect in T-cell function in HAM/TSP and contrasts with the findings in MS in which reduced CTL to viruses other than measles has not been observed.

The mechanisms responsible for reduced measles virus CTL in MS have not been established. Because measles-specific CTL are CD4<sup>+</sup>, the possibility was considered that reduced measles virus CTL in MS reflects a defect in this T-cell subset. CD4<sup>+</sup> CTL specific for mumps virus are normal in MS and does not support the concept of a generalized CD4<sup>+</sup> abnormality. A second possibility that the reduced measles virus CTL in MS is due to sequestration in the CNS of CTLs that cross-react with CNS antigens has been examined. The peptide specificity of CTL to individual measles virus polypeptides in patients with MS as well as normals has been assessed. In both groups a measles virus-specific CTL reaction was found with at least two different proteins. Since it is unlikely that shared epitopes between more than one measles protein and CNS components exist, the observations do not support the conclusion that the reduced CTL in MS reflects cross-reactivity with CNS antigens. The hypothesis currently favored by the CIS is that the reduced CTL to measles virus in MS is related to decreased generation, or maintenance of this immunological reaction. Accordingly, the mechanisms involved in the regulation of T-cell reactivity to measles virus antigens is being studied.

During FY 1989, the assessment of genetic components related to MS have continued. Previous findings, including an increased familial prevalence of the disease and a higher concordance in monozygotic than dizygotic twins, provide evidence for a genetic component to the pathogenesis of MS. Because immune reactivity is regulated by the MHC (HLA in humans), there has been extensive study of HLA molecules in the disorder. Associations between MS and certain serotypes of HLA class II HLA molecules serotype are well-known. These were updated during FY 1989, and DR2 and DQw1 were found in approximately 60% and 78%, respectively, of the NIB, MS patients. In an initial study of genes encoding class II MHC molecules, in collaboration with Dr. Jack Strominger at Harvard Medical School, correlation between MS and an RFLP associated with the Dw2 subgroup of DR2 was identified. Other investigators identified linkage with a genetic fragment encoding DQw1. In order to pursue these leads, genes encoding DQB genes have been cloned from normal homozygous typing cells and from homozygous MS patients. During FY 1989, these were sequenced but significant differences were not identified. In additional studies, focus was placed on exons that encode polymorphic portions of class II MHC genes. Using the polymerase chain reaction (PCR) and specific oligonucleotide primers, DQA, DQB, and DRB sequences have been amplified, cloned, and sequenced. To date, a total of 60 gene sequences from a panel of five MS patients with different DR specificities have been compared with known sequences of class II genes and significant differences have not been identified. These observations have led to the conclusion that in MS, in contrast to insulin-dependent diabetes mellitus, a unique class II HLA gene that is related to susceptibility or resistance, is unlikely.

Recent findings from a number of laboratories indicate that expression of MHC varies in different tissues, including the CNS. During FY 1989, major efforts have been initiated to study the control of MHC in the CNS. Human glioblastoma multiforme cell lines, primary cultures of adult human glia cells (AHGC), neonatal murine brain cells, and skin keratinocytes are being studied. A recent demonstration that regulation of class II MHC molecules in rat keratinocytes parallels that seen in astrocytes. Glioblastoma cell lines that have been established and characterized by Dr. Darryl Bigner, Professor of Neuropathology, Duke University, are being used to assess the expression of class II MHC molecules. Preliminary studies have been conducted with U 105. This cell line expresses both class I and HLA DR molecules but does not constitutively express HLA DQ molecules. Experiments in the NIB have demonstrated that expression of HLA DQ is induced by treatment with interferon gamma, but this effect is downregulated by IL1. The level of DQ expressed on the cell surface is paralleled by the amount of specific RNA which indicates that the regulatory effects are exerted at transcription. The molecular mechanisms are being analyzed with genetic constructs that bind to particular regulatory elements. The experimental approaches developed with the U 105 cells will be extended to the study of additional glioblastoma cell lines, neuronal cell lines, and brain capillary endothelial cells, in collaboration with Dr. Richard McCarron in LNNS. The regulation of class II MHC molecules is also being studied in keratinocyte cultures from skin biopsies of patients with MS and normal controls.

Parallel studies are being conducted with AHGC. The expression of both class I and class II HLA MHC molecules on AHGC is upregulated by gamma



interferon but is unaffected by infection of these cells with viruses, such as herpes simplex or influenza. Astrocytes that are infected with influenza can function as targets for influenza-specific CTL. In addition, gamma interferon treated AHGC cocultured with MBP can be lysed by CD4<sup>+</sup> MBP-specific CTL. Collectively, these findings demonstrate that adult astrocytes can participate as antigen-presenting cells for T-cell recognition.

Two major findings have emerged from studies of MHC molecules in newborn murine astrocyte cultures. First, the expression of class I MHC molecules on astrocytes is different from other cells, namely macrophages. Secondly, the spontaneous and induced expression of class I MHC molecules varies among inbred strains of mice. Mapping studies with recombinant inbred strains have provided evidence that the genes responsible for these differences reside in the MHC complex. The surface expression of class I MHC molecules correlates with specific mRNA indicating a transcriptional control mechanism. Experiments have been initiated to identify the genetic mechanisms responsible for these irregularities in collaboration with Dr. Keiko Ozato, Chief, Molecular Genetics of Immunity Section, NICHD.

As indicated above, it is generally accepted that more than one genetic element is involved in susceptibility to MS, and during FY 1989, findings in the MIS that link MS to a gene encoding the V-beta region of the T cell receptor (TcR) were published. In these studies, the TcR germline DNA of 40 chronic-progressive MS patients and 100 normal individuals were compared with probes representing 14 different human V-beta subfamilies. No differences in the number of gene segments defined by these probes were found; however, the distribution of haplotypes identified by RFLP alleles detected with V-beta 8, V beta-11, and C-beta probes of MS patients was significantly different from that found in a normal population of 100 individuals ( $P < .012$ ). Because 84% of the MS patients were DR2<sup>+</sup> Caucasians, the findings were compared to a second control population consisting of 43 DR2<sup>+</sup> Caucasians. The haplotype differences were even more highly significant ( $P < .003$ ). These data indicate the existence of an MS susceptibility gene associated with the TcR beta chain gene complex. These initial studies have been expanded and as additional patients have been studied, the findings have reached greater statistical significance. These investigations are being extended into families with more than one affected member. In addition, probes for TcR alpha chain genes are being used. Collectively, the findings support the concept that MS is associated both with genes that encode for MHC molecules that are involved in antigen presentation, and TcR genes that encode for antigen recognition.

A second major project in the MIS involves the investigation of antigen presentation by class I HLA molecules. A model system has been established for the generation of HLA-A2.1-restricted human CTL that recognize the influenza virus matrix peptide M1 55-73. Initial experiments using a panel of seven amino acid sequence variants of the M1 peptide demonstrated that all of these variants can bind to HLA-A2.1 but only five of the seven are recognized by CTL. Subsequent experiments examined the capacity of 24 unrelated peptides to compete with M1 55-73 for presentation by HLA-A2.1. Five of these unrelated peptides showed significant competition in the assay system. All of these contain three adjacent hydrophobic amino acids which suggests that this structural feature may be critical for peptide interaction with the HLA-A2.1 molecule.

The structural contributions of HLA-A2.1 are also being examined. Two amino acid positions in the floor of the peptide binding groove and three on the alpha 2 helix that form part of the peptide binding groove have been found to affect antigen presentation of the M1 peptide. Most of these amino acid substitutions affect presentation without inhibiting the binding of the M1 55-73 peptide. In ongoing experiments on relationships between structure and function of nonconserved amino acids in the floor of the peptide binding groove are being studied with a series of site-directed mutants of HLA-A2.1. This approach should further characterize the structural features of this area and the binding site involved in presentation of antigen to CD8<sup>+</sup> T cells.

Other fundamental studies in the MIS are examining the mechanisms by which cytosolic viral peptides are processed and interact with class I MHC molecules. One obvious intracellular location for such a process is the endoplasmic reticulum, where proteins are translocated across the membrane into the secretory system. The antibiotic Brefeldin A blocks the movement of proteins out of the endoplasmic reticulum and is being used to study this fundamental question. Treatment of virus exposed targets with Brefeldin A blocks the capacity of targets to be recognized by viral specific HLA-A2.1 restricted CTL even though viral protein synthesis is not inhibited. This implies that egress from the endoplasmic reticulum is required for viral antigen presentation by class I MHC molecules at the cell surface. These investigations are being extended to study antigen presentation by class II MHC molecules to determine if the pathways for endogenous antigen presentation are similar for both class I and class II molecules.

During FY 1989, considerable effort was directed at the study of immune function in HAM/TSP and to similar studies in MS. In initial studies of nine patients with HAM/TSP, a number of immunological abnormalities were detected. Although the numbers and distributions of blood lymphocytes are normal, there was increased expression of surface molecules, such as a receptor for interleukin 2, that are consistent with activation. Lymphocytes from these patients also have the tendency to undergo spontaneous proliferation, and as mentioned above, there is a defect in the generation of virus-specific CTL. These findings were postulated to be related to HTLV-I infection. During FY 1988, new methods were developed for the detection of HTLV-I in lymphocytes, and to date, the virus has been demonstrated in 16 of 18 cell lines derived from the blood or CSF of the 9 patients. DNA from such cell lines have been studied by Southern blot hybridization using a number of restriction enzymes and a full-length HTLV-I probe. Although the findings are consistent with HTLV-I, restriction maps of the viruses obtained from some patients are different. The total proviral sequence of one isolate from HAM/TSP has been obtained in collaboration with Dr. Ashley Haase, Chairman of Microbiology, University of Minnesota. This showed 97% homology with conventional HTLV-I. One of the nucleotide difference was a point mutation that resulted in a premature stop code in the gene encoding the envelope protein. Other isolates are being examined to determine if this change represents a unique variant in one patient or is representative of the viruses associated HAM/TSP.

Other differences between viruses associated with HAM/TSP and prototype HTLV-I have been observed. A prominent feature of T-cell lines such as HUT 102 or MT 2 infected with prototype HTLV-I is that they are transformed (IL2 independent cell lines). This property of transformation is believed to be a

unique feature of HTLV-I and has been the basis of studies on the capacity of this agent to induce leukemogenesis. In comparison, the HAM/TSP is not a malignant disorder, and the T-cell lines derived from HAM/TSP have remained IL2 dependent and have not become transformed. Studies are in progress to identify molecular differences which would account for these biological findings.

As indicated above, a retrovirus is an attractive candidate for an etiological agent for MS. Recent studies from at least three laboratories have described the presence of HTLV-I-related viral sequences in amplified DNA from patients with MS. Experiments in the NIB using similar primers and probes have been conducted. Initial studies have indicated that HTLV-I-related sequences can be easily detected in DNA from patients with HAM/TSP. In addition, studies of HTLV-I-seropositive normal individuals conducted in collaboration with Dr. William Blattner, Chief, VES, NCI, has shown that most of these seropositive individuals have lymphocytes that undergo spontaneous proliferation in tissue culture and contain HTLV-I sequences. However, to date, efforts to detect HTLV-I-related sequence in the vast majority of MS patients have been unsuccessful. In a small number of patients, DNA that reacts with an HTLV-I POL region probe has been amplified. Confirmation of these findings will require complete nucleotide sequence analysis of the hybridizing bands.

A complementary approach involves assessment of the T-cell response to HTLV-I. In these experiments, CTL that lyse autologous HTLV-I infected cells have been generated, and it has been shown that these recognize an epitope residing in the envelope region. It is of considerable interest that this same epitope reacts with antibody to HTLV-I. In most viruses the epitopes that react with T cells and antibody are different. Another exception is HIV-1 which contains an immunodominant epitope that reacts with CTL as well as antibody. These findings suggest that a unique feature of human retroviruses is that both cell-mediated and antibody components react with similar epitopes.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02202-14 NI

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies in Patients with Multiple Sclerosis and Other CNS Diseases

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                                  |                      |    |     |       |
|----------------------------------|----------------------|----|-----|-------|
| PI: Dale E. McFarlin, M.D.       | Chief                | NI | DIR | NINDS |
| Others: Henry F. McFarland, M.D. | Deputy Chief         | NI | DIR | NINDS |
| Steven Jacobson, Ph.D.           | Senior Staff Fellow  | NI | DIR | NINDS |
| Ajay Gupta, M.D.                 | Visiting Associate   | NI | DIR | NINDS |
| David Mattson, M.D.              | Medical Staff Fellow | NI | DIR | NINDS |
| Elliot P. Cowan, Ph.D.           | Senior Staff Fellow  | NI | DIR | NINDS |

## COOPERATING UNITS (if any)

E. Alexander, M.D., Assoc. Prof. Dept. of Med., Johns Hopkins University; L. Hood, M.D., Chairman, Dept. of Biology, Cal. Institute of Technology; Steven Beall, M.D., Cal. Institute of Technology

## LAB/BRANCH

Neuroimmunology, CNP

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

12.0

## PROFESSIONAL:

8.0

## OTHER:

4.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The broad goal of this project is to obtain an understanding of the multiple factors related, either singly or in combination, to the pathogenesis of neurological diseases. Focus is currently placed on multiple sclerosis (MS). Particular attention is being given to genetic and immunological factors. Studies of genetic influence include examination of genes encoding for HLA molecules and the T-cell receptor. These studies are performed in patients with sporadic disease, members of families with multiple affected members, and identical or nonidentical twins either concordant or discordant for MS.

Parallel studies are being conducted in diseases which have a clinical presentation similar to MS. A chronic myelopathy known as HTLV-I-associated myelopathy/tropical spastic paraplegia (HAM/TSP) is being studied clinically and immunologically. Other diseases affecting cerebral white matter such as Sjogren's disease and familial leukodystrophy are being examined by neuroimaging techniques.

A second general goal is to conduct trials of experimental therapeutic agents. Analysis of data from a double-blind phase III trial of cyclosporine A for the treatment of MS indicate that patients treated with this agent progress less rapidly than patients who received placebo; however, a portion of patients developed renal toxicity. Open-label studies on the relationship between cyclosporine A treatment and nephrotoxicity are being conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02203-14 NI

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Membrane Molecules Involved in Immune Reactivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                                  |                      |      |     |                    |
|----------------------------------|----------------------|------|-----|--------------------|
| PI: Dale E. McFarlin, M.D.       | Chief                | NI   | DIR | NINDS              |
| Others: Henry F. McFarland, M.D. | Deputy Chief         | NI   | DIR | NINDS              |
| Steven Jacobson, Ph.D.           | Senior Staff Fellow  | NI   | DIR | NINDS              |
| Suhayl Dhib-Jalbut, M.D.         | Visiting Associate   | NI   | DIR | NINDS              |
| Ajay Gupta, M.D.                 | Visiting Associate   | NI   | DIR | NINDS              |
| Steven J. Greenberg, M.D.        | Medical Staff Fellow | DCBD | MET | NCI                |
| Cedric Raine, Ph.D.              | Professor            |      |     | Albert Einstein U. |

COOPERATING UNITS (If any)

Dr. William Blattner, Chief, VES, NCI; Dr. Ashley Haase, Chairman, Dept. of Microbiology, University of Minnesota; Dr. Thomas Waldmann, Chief, MET Branch, NCI

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

2.5

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to assess the expression and function of membrane molecules on immune cells and to characterize viral antigens which are targets of the immune response.

The research is focused on immune reactivity to measles virus and human T cell lymphocytotropic virus I (HTLV-I). Immune responses to the individual components of these viruses are being examined in patients with neurological diseases. Antibodies in the blood and the CSF are measured by ELISA. Cellular immune responses are determined by lymphocyte proliferation and cytotoxicity. Purified viral proteins are prepared by procedures developed in our laboratory and used in these studies.

The expression of various molecules on the outer membrane surface is assessed on lymphocytes from patients with neurological disorders. This research is currently focused on HTLV-I-associated myelopathy/tropical spastic paraplegia, (HAM/TSP) a chronic neurological disease associated with HTLV-I, but parallel studies are also being conducted in other disorders. The expression of HTLV-I genome in lymphocytes from patients with HAM/TSP, normal carriers of HTLV-I, and multiple sclerosis is being evaluated. The effect of HTLV-I infection on immune function is being studied.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02204-14 NI

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Mechanisms  
Operative in Experimental Autoimmune Diseases of the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                                 |                     |                    |     |        |
|---------------------------------|---------------------|--------------------|-----|--------|
| PI: Dale E. McFarlin, M.D.      | Chief               | NI                 | DIR | NINDS  |
| Others: Richard McCarron, Ph.D. | Senior Staff Fellow | LNNS               | DIR | NINDS  |
| Robert Fallis, M.D.             | Senior Staff Fellow | NI                 | DIR | NINDS  |
| Paul Massa, Ph.D.               | Senior Staff Fellow | NI                 | DIR | NINDS  |
| Cedric Raine, Ph.D..            | Professor           | Albert Einstein U. |     |        |
| Keiko Ozato, Ph.D.              | Section Chief       | LDMI               | DIR | NICHHD |
| Maria Spatz, M.D.               | Section Chief       | LNNS               | DIR | NINDS  |

## COOPERATING UNITS (if any)

## LAB/BRANCH

Neuroimmunology, CNP

## SECTION

Neurological Diseases Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.5

## PROFESSIONAL:

2.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The mechanisms responsible for the production of experimental allergic encephalomyelitis, a model of autoimmune disease are being examined. Current research is focused on an adoptively transferred model which is produced by the transfer of lymphocytes sensitized against myelin basic protein in syngeneic animals. Under optimal conditions, neurological dysfunction occurs; this is characterized pathologically by inflammation and primary demyelination. Many mice recover and develop chronic-relapsing disease. The mechanisms responsible for both the initial and the chronic disease are not known, but an early event is the migration of immune cells across the blood-brain barrier into the central nervous system. This is formed by the capillary endothelial cells. Other cells in close proximity are astrocytes and microglia. Antigen presentation by macrophages, capillary endothelial cells in the brain, and astrocytes are being compared. The expression of MHC molecules on these cells is also being evaluated. An encephalitogenic epitope for SJL mice has been identified and T-cell lines against this have been derived. These ootn proliferate and show cytotoxic activity.

|   |   |   |
|---|---|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |   | <b>PROJECT NUMBER</b><br>Z01 NS 02205-14 NI   |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989   |   |   |
| <b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b><br>Interaction Between Viruses and the Host Immune System  |   |   |
| <b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b>  |   |   |
| PI: Henry F. McFarland, M.D.<br>Others: Dale E. McFarlin, M.D.<br>Steven Jacobson, Ph.D.<br>William E. Biddison, Ph.D.<br>Snari de Silva, M.D.<br>Suhayl Dhib-Jalbut, M.D.<br>Eric Long, Ph.D.  | Section Chief,<br>Chief<br>Senior Staff Fellow<br>Section Chief<br>Medical Staff Fellow<br>Visiting Associate<br>Visiting Scientist | NI DIR NINDS<br>NI DIR NINDS<br>NI DIR NINDS<br>NI DIR NINDS<br>NI DIR NINDS<br>NI DIR NINDS<br>BRB DIR NIAID |
| <b>COOPERATING UNITS (if any)</b><br>John R. Richert, M.D., Assist. Prof., Georgetown University; Diane Griffin, M.D., Ph.D., Prof., Dept. Neurology, Johns Hopkins University; Richard Johnson, M.D., Chairman, Department Neurology, Johns Hopkins University   |   |   |
| <b>LAB/BRANCH</b><br>Neuroimmunology, CNP   |   |   |
| <b>SECTION</b><br>Cellular Immunology Section   |   |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, Maryland 20892   |   |   |
| <b>TOTAL MAN-YEARS:</b><br>4.5  | <b>PROFESSIONAL:</b><br>4.0   | <b>OTHER:</b><br>0.5  |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither<br><input type="checkbox"/> (a1) Minors<br><input type="checkbox"/> (a2) Interviews  |   |   |
| <b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b><br><p>             The purpose of this project is to evaluate the immune response to viruses in healthy individuals and to identify abnormalities in these responses which may be related to <u>disease of the nervous system</u>, particularly, <u>multiple sclerosis (MS)</u>. These studies focus on functional analysis of the <u>cellular immune response</u> to viruses which commonly affect humans and which may be involved in diseases of the nervous system. Emphasis is on identification of the mechanisms of reduced generation of measles virus-specific <u>cytotoxic T cells (CTL)</u> in patients with MS. The mechanisms involved in the regulation of these responses are being examined. An additional goal of these studies is to evaluate the influence of <u>genetic makeup</u> on both induction and effector phases of the immune response to viruses. CTL and <u>helper T cells</u> are assayed. These studies use techniques of molecular biology to incorporate the genes for HLA class II molecules into appropriate target cells in order to examine specific restriction elements. Virus antigens recognized by these cell populations and the influence of antigen presentation on the generation of these responses are being examined. The possibility that viral infections may influence the immune response to <u>antigens of the central nervous system</u> such as <u>myelin basic protein</u> is also being examined. In addition, the capacity of glial cells derived from adult human brain to process and present viral antigens to immune T cells is being examined in order to help understand the effects of viral-specific T cells on viral infections of the nervous system. A major component of these studies is to examine the effect of viruses and various <u>lymphokines</u> on the regulation of HLA molecules on glial cultures.           </p> |   |   |
| 10 - NIB/DIR  |   |   |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

201 NS 02603-06 NI

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Lymphoid Cell-Cell Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William E. Biddison, Ph.D. Section Chief NI DIR NINDS

Others:

|                               |                      |     |     |       |
|-------------------------------|----------------------|-----|-----|-------|
| David H. Mattson, M.D., Ph.D. | Medical Staff Fellow | NI  | DIR | NINDS |
| Naoki Shimojo, M.D.           | Visiting Fellow      | NI  | DIR | NINDS |
| Bronya Shvetsky, M.D.         | Visiting Scientist   | NI  | DIR | NINDS |
| Elliot P. Cowan, Ph.D.        | Senior Staff Fellow  | NI  | DIR | NINDS |
| W. Lee Maloy, Ph.D.           | Senior Investigator  | BRB | DIR | NIAID |
| John E. Coligan, Ph.D.        | Chief                | BRB | DIR | NIAID |

## COOPERATING UNITS (if any)

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## LAB/BRANCH

Neuroimmunology, CNP

## SECTION

Molecular Immunology Section

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

6.0

## PROFESSIONAL:

3.0

## OTHER:

3.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general objective of this project is to define the mechanisms by which human lymphoid cells interact with each other and with foreign antigen-bearing cells in order to produce and regulate immune responses. Over the past year, there have been two major efforts underway that are targeted on this objective: 1) dissection of the molecular basis for T cell recognition of viral antigens presented by HLA class I molecules; and 2) analysis of the potential role of human T cell receptor (TcR) beta chain genes in susceptibility to multiple sclerosis (MS). The principle findings are as follows: 1) amino acid side chains on the alpha two helix and beta sheet floor of the peptide binding groove of HLA-A2 can determine the form of the peptide-A2 complex that is recognized by T cells; 2) approximately 25% of randomly selected synthetic peptides can bind to the HLA-A2 molecule as assessed by a competition assay; 3) a common structural feature of peptides that can bind detectably to HLA-A2 is that they all have a stretch of three adjacent hydrophobic amino acids; 4) presentation of an internal viral antigen by HLA-A2 in virus-infected cells requires that the viral antigen transit the endoplasmic reticulum -> golgi pathway in order to be recognized on the cell surface by T cell receptors; and 5) a significant difference between MS patients and normals was found in the distribution of the haplotypes defined by using V-beta 8, V-beta 11, and G-beta probes, indicating that at least one set of genes that influence susceptibility to MS is located in the region of the TcR beta chain complex.









**ANNUAL REPORT**  
October 1, 1988 through September 30, 1989

Surgical Neurology Branch - Clinical Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke  
Edward H. Oldfield, M.D., Chief

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October 1, 1988 through September 30, 1989  
Surgical Neurology Branch, Division of Intramural Research  
Clinical Neurosciences Program  
Edward H. Oldfield, M.D., Chief

National Institute of Neurological Disorders and Stroke

## SUMMARY OF STUDIES

The Surgical Neurology Branch (SNB) principal activities are conducted in the following: 1) Clinical Neurosurgery Section; 2) Biochemistry Section; 3) Central Nervous System Implantation Unit; 4) Tumor Biology Unit; 5) Molecular Biology Unit.

The SNB has as its major research functions: (1) the study of the biology and of therapeutic approaches to malignant and benign tumors of the brain, and pituitary gland; (2) investigation of the capability of implanted tissues into the brain of animals and humans to survive and integrate anatomically or biochemically with the host brain to influence brain function; (3) the investigation of certain vascular disorders of the CNS; and (4) the investigation and surgical treatment of patients with epilepsy. Its clinical function is to provide the neurosurgical services to its own research protocol patients and to patients seen in consultation in the NIH, Clinical Center. The SNB is presently located in Buildings 9 and 10. Its staff includes 15 clinical neurosurgeons at various levels of training and experience, as well as 1 senior scientist, 3 junior scientists and a support staff of technical and administrative individuals.

In addition to its primary functions of clinical and basic research, the SNB provides young neurosurgeons with experience in clinical and laboratory investigation in a combined clinical and neuroscience environment. Of those who have participated in the SNB program as Medical Staff Fellows and Senior Staff Fellows, many have entered academic positions in neurosurgery.

The following significant observations have been made during the past 24 months. In the area of brain tumors we have made several observations. Using PET scanning and positron-labeled fluoro-2-deoxy-glucose, we demonstrated that thiopental selectively suppress brain metabolism by approximately 50% while leaving tumor metabolism unaffected. Similar, but less suppression was demonstrated with sedative doses of pentobarbital. Means to exploit this capacity to selectively influence the brain metabolism without effecting tumor metabolism for therapy are currently under investigation. This technique is also a method of functional metabolic "contrast enhancement" for PET and should be applicable to enhance the demonstration of other, non-tumorous, lesions of the brain.

We then demonstrated in rats that early and delayed cerebral radiation toxicity is prevented by pretreatment with pentobarbital. Survival of animals receiving whole brain irradiation was increased from 0-20 to 45-70% by sedation with pentobarbital just before irradiation exposure. The mechanism of this protection was investigated and shown to be linked to interaction of barbiturates with the GABA receptor. Other agents which functionally bind the GABA receptor were shown to be equally effective. Since tumors lack GABA receptors, and tumor metabolism had been shown not to be influenced by barbiturates, animals with implanted tumors were investigated. No reduction of tumor sensitivity was demonstrated. Hence, pentobarbital suppresses metabolism of normal brain and

protects against radiation toxicity, but tumors have no metabolic suppression and are not protected against irradiation injury. Experiments with subhuman primates and patients are underway.

Further progress was made in the development of immunotoxins for treatment of CNS tumors. The major goal of this project is to develop immunotoxins which will selectively kill tumor cells *in vivo*. An antitransferrin receptor antibody-toxic peptide conjugate was created against the transferrin receptor which specifically kills tumor cells *in vitro* and also when injected into the cerebrospinal fluid. A cloned diphtheria toxin was created in which the C-terminal 15kD region was deleted. This eliminated much of the nonspecific binding of the diphtheria toxin, but retained the entry function of the toxin into the cytosol of cells and retained toxicity. Recent results indicate that point mutations can increase selective cytotoxicity of antibody-toxin conjugates up to 10,000-fold. Animal studies indicated a 5-log kill of tumor cells inoculated into the peritoneal cavity *in vivo*. Utility of the immunotoxins in animal models of leptomeningeal tumor was demonstrated. Treatment of tumor in a guinea pig model of leptomeningeal neoplasia demonstrated a 2 to 3-log, and in some animals a 5-log kill of tumor cells in the central nervous system with this conjugate. Clinical trials in patients with carcinomatous meningitis will begin this year. Collaborative efforts between the Biochemistry Section of SNB and a bone marrow transplant team at the University of Minnesota using immunotoxins to purge the implanted marrow of T cells resulted in more rapid marrow engraftment, reduced hospitalization, and diminished graft versus host disease in the 24 treated patients.

Several investigations were completed of adoptive immunotherapy for treatment of malignant brain tumors. Techniques were perfected to successfully and implant reproducible lymphokine activated Killer (LAK) cells into the brain of rodents and primates. Clinical investigations of adoptive immunotherapy using interleukin-2 (IL2) and LAK against cerebral gliomas indicated that repeated injections of IL2 and LAK into tumor cavities or directly into brain tumors produced increased cerebral edema and transient or permanent new neurological deficits in all patients. Investigation of intravenous IL2 therapy in patients with glioblastomas demonstrated increased edema from the tumor bearing region. The results of these studies indicate that adoptive immunotherapy using IL2 and LAK cells produces significant toxicity and rarely elicits a measurable tumor response. In separate clinical studies IL2 was (1) demonstrated to penetrate the blood-CSF barrier in patients without CNS tumor involvement in sufficient concentration to maintain activity of LAK cells in the CSF and (2) using MRI scanning, IL2 was shown to increase the water content of the normal brain, suggesting alteration of the normal blood-brain-barrier by IL2. Animal experiments indicated that the cerebral edema results from IL2-induced increased vascular permeability in the vessels of the tumor and the surrounding brain. It was shown in an animal model of cerebral metastases that, although lung metastases responded to IL2/LAK treatment, brain deposits of the same tumor in the same animal had no response to treatment, indicating that the blood-brain-tumor barrier restricts effectiveness of immunotherapy of this type. The requirement of continued exposure of LAK cells to IL2 for maintenance of optimal cytotoxicity was demonstrated in an *in vitro* analysis of LAK cytotoxicity against glioma cells. Intraarterial infusion of IL2 was shown to increase delivery of IL2 to brain by 20-fold compared to intravenous infusion.

Electron microscopy demonstrated that the interaction of lymphokine activated killer cells with malignant brain tumor cells, which produces cytotoxicity, was based on the local exocytosis of granules or vesicles by the effector cell into the



small extracellular space between the effector/target cell conjugate. This is a common cytotoxic mechanism for cytotoxic T cells, natural killer cells, and LAK cells against tumor cell targets.

That drug streaming, which is responsible for toxicity and limits efficacy after intraarterial infusion of chemotherapy, can be eliminated in humans by phased diastolic delivery was demonstrated in humans using PET scanning and positron-labeled water to demonstrate the distribution and to quantify the delivery of infusate to the brain after intracarotid delivery. Patients treated with intracarotid cisplatin using a prototype pump which delivers drug into the internal carotid artery only during a brief period of diastole, so that drug streaming is eliminated, had no retinal or neural toxicity related to drug streaming. A trial of intraarterial high dose BCNU via phased diastolic delivery was initiated in patients with malignant glioma and continues with the goal of minimizing neural toxicity due to this agent.

Using intraarterial infusion of cisplatin combined with extracorporeal circulation of the jugular venous blood, we achieved nearly complete vascular isolation of the brain. Therefore, the cerebral circulation can be isolated by the rather simple means of two transcutaneous catheters, one in the carotid artery and the other in the ipsilateral jugular vein. Extracorporeal hemodialysis of the ipsilateral jugular blood removes as much as 90% of the cisplatin infused into the internal carotid artery before this drug reaches the systemic circulation and sensitive normal tissues are exposed.

A protein that is secreted by malignant brain tumors and increases vascular permeability to macromolecules has been isolated from the incubation media from malignant brain tumors. We have demonstrated that this material is a protein of approximately 45,000 daltons molecular weight and that its secretion is eliminated by incubation of the tumor cells with dexamethasone. Further experiments indicate that it binds a heparin-like moiety on the cell surface of endothelial cells and that influx of  $\text{Ca}^{+2}$  into the endothelial cell occurs in response to this interaction. Certain divalent cations were shown to inhibit the response of endothelial cells to this tumor-derived vascular permeability factor. These observations explain the origin of brain edema in patients with brain tumors and may permit development of therapy against tumor-associated cerebral edema, which now requires high dose glucocorticoid therapy and is associated with significant complications. Also, we have shown that, using the PET scan and gallium-EDTA to study patients with malignant brain tumors before and after steroid therapy, diminished interstitial fluid volume occurs in response to treatment with dexamethasone.

Insulin and the insulin-like growth factors 1 and 2 (IGF 1 and IGF 2) were investigated for a role in the growth of malignant gliomas. High levels of IGF-1 receptors were demonstrated on several gliomas and shown to be functional as determined by autophosphorylation of the receptor and DNA synthesis. These results suggest a role of IGF-1 and its receptor in the regulation of the growth of gliomas.

Investigation of interstitial therapy of malignant tumors with radioactive seeds has shown nearly consistent response of tumors, but an appreciable incidence of local radiation-induced toxicity. Toxicity has been demonstrated to be related to the dose administered, the rate of administration and the volume of treatment. This form of therapy for local delivery of high doses of irradiation prolongs survival in many patients with malignant tumors, particularly those that are small and have focal involvement.

The clinical, genetic and radiographic features of patients with symptomatic von Hippel-Lindau disease (vHL) was investigated. In collaboration with Drs. Bert Zbar and Marston Linehan of NCI and Dr. Michelle Filling-Katz of NINDS, we investigated the genetics of 9 tumors from 5 patients with von Hippel-Lindau (vHL) disease and hemangioblastomas of the brain and spinal cord. The tumors were shown to have absence of the wild type (normal) allele and were shown to arise by a mechanism analogous to that of familial retinoblastomas. That is, by homozygous absence of a tumor suppressing gene. MRI scanning was used to demonstrate the extent of the sphingomyelia cavity in patients with hemangioblastomas of the spinal cord. MRI with Gadolinium-EDTA contrast enhancement was used to investigate the brain and spinal cord in vHL disease and shown to be more sensitive than MRI without Gd-EDTA and more sensitive than CT scanning, but not as sensitive as arteriography, for tumor detection. The sphingomyelia that is associated with about 90% of hemangioblastomas of the spinal cord, and which is responsible for the neurological deficit in many of these patients, was shown to resolve with treatment directed at complete tumor removal and not to require separate treatment.

Further experience with selective pituitary venous sampling for Cushing's syndrome indicated that selective bilateral simultaneous inferior petrosal sinus sampling successfully distinguishes patients with Cushing's disease from those with ectopic ACTH syndrome with greater than 99% accuracy. This technique permits tumor localization within the pituitary gland in patients with Cushing's disease with about 85% accuracy. It was also demonstrated that repeat surgery could be used to successfully treat (70% cure rate) patients with persistent or recurrent Cushing's disease after prior pituitary surgery or pituitary irradiation. Predictive features of successful outcome and guidelines for intraoperative management were established by this study of 29 patients with repeat surgery. Gadolinium-EDTA enhancement with MRI scanning was shown to be superior to current imaging techniques for demonstration of the site of pituitary microadenomas in Cushing's disease pre-operatively. The critical aspect of timing the image procurement within 5-10 minutes after the injection of Gd-EDTA was demonstrated.

Encouraging progress was made in the area of implantation of tissues from the central nervous system into the host brain in primates. Initially, a model of Parkinsonism which affects only one-half of the brain was developed by infusing MPTP into the internal carotid artery. Using this model of hemiparkinsonism and the previously available model of MPTP-induced full Parkinsonism in the primate, we investigated functional recovery and graft survival after implanting dopaminergic tissues into the caudate nucleus of lesioned animals. Both adrenal and fetal substantia nigra allografts were demonstrated to induce partial functional recovery in the host animals. However, the recovery was much more pronounced using fetal substantia nigra for implantation. Immunohistological assessment demonstrated functional survival of the fetal grafts and prominent sprouting of dopaminergic fibers from resident fibers in the host brain surrounding the area of implantation. These results indicate that sprouting of the intrinsic dopaminergic pathways of the host brain is induced by either the surgical cavitation or the fetal implants. If the basic mechanisms responsible for this induced sprouting can be identified and used for other neuronal systems, practical application of central nervous system implants for Parkinson's disease and other CNS disorders may be possible. The sprouting in response to cavitation and implantation which appeared to be related to functional recovery of the animals appeared to derive from the ventral tegmental tract, a dopaminergic neural tract which is separate from the nigrostriatal tract and which is not affected by MPTP. To study this phenomenon in greater detail, and without the

requirement of large numbers of subhuman primates, a rat model of Parkinsonism was developed with preservation of the ventral tegmental tract. In addition, accumulation of a material which induces neural sprouting in an *in vitro* assay was found in the cavities of monkeys and humans after surgical cavitation. Finally, a technique was developed to assess the biochemistry of the basal ganglia of implanted primates *in vivo* by implanting fine dialysis fibers into the basal ganglia. This technique should not only be rewarding for investigation of implantation to tissues to reverse Parkinsonism, but should have wide application for clinical research. We developed a model in which transplantation of fetal rat pituitary tissue, with and without hypothalamus, into hypophysectomized rats was accomplished successfully. There is evidence of peripheral plasma levels of anterior pituitary hormones and surviving anterior pituitary cells in this model.

Retrospective assessment of 81 patients with spinal AVMs indicated that there are 2 major types of AVMs affecting the spine, arteriovenous fistulas and true AVMs of the spinal cord. Distinct clinical syndromes were identified to be associated with each of these types of AVMs, different mechanisms of the production of myelopathy were clearly identified, and demographic considerations indicate that spinal dural AV fistulas are acquired, rather than congenital in origin. Three patients were identified and treated in whom the apparent diagnosis was a spinal AVM but each of whom had a cranial dural AV fistulas which drained intradurally into the venous system of the spinal cord. These findings established that venous congestion alone can produce myelopathy and that the myelopathy of venous congestion is reversible. They also indicate that other disease processes may produce myelopathy by a similar, although previously unrecognized, mechanism. Our experience with five patients with Foix-Alajouanine syndrome indicates that these patients have myelopathy from venous congestion and that, if they are diagnosed and treated early, the myelopathy is amenable. Therefore, they do not necessarily suffer a result of venous thrombosis and thus are not responsive to treatment, as has been previously thought. Embolic occlusion has been considered definitive treatment of spinal AVMs which can be completely obliterated. We sequentially investigated 6 patients after embolic occlusion of spinal AVMs and found that 5 of the 6 had recanalized and caused clinical relapse within the first year following embolization. Our results indicate that embolization for spinal AVMs is only temporarily effective therapy.

Finally, the investigation of the mechanism and physiological role of programmed cell death, which occurs in both the central nervous system and the immune system during normal development, demonstrated that thymocytes can be induced to die by the same signal that stimulates mature T cells to proliferate. RNA and protein synthesis inhibitors which block new gene expression interrupted the programmed cell death. This is consistent with a new model of immune system self tolerance that states that potentially autoreactive cells are induced to die by crosslinking the T cell receptor early in development, the same signal that induces mature T cells to react against foreign antigens. Studies are being extended into the nervous system by examining the mechanism of glutamate-induced neuron death *in vitro* and *in vivo*. A new immunotoxin induced selective purkinje cell death in an animal model has been developed in our Branch which is being used to examine the possible mechanism(s) of purkinje cell death in a variety of neurological disorders.

A Molecular Biology Unit has been established within the Branch during the past year in order to study genetic abnormalities associated with various nervous system disorders, especially primary brain tumors.



## I. CLINICAL NEUROSURGERY SECTION

Edward H. Oldfield, M.D., Chief

The clinical activities of the Surgical Neurology Branch are primarily directed to the investigation of the biological behavior and mechanisms of pathophysiology of malignant primary brain tumors, pituitary tumors, certain vascular disorders of the CNS, the surgical management of epilepsy refractive to medical therapy, and the investigation of tissue implantation in the CNS to reverse neurological disorders.

### A. Brain Tumors

#### 1. The Use of Barbiturate Sedation in the Management of Brain Tumors

##### Evaluation of the Influence of Compounds Which Antagonize the GABA-mimetic Properties of Pentobarbital on its Radioprotective Effect

Pentobarbital has been shown to have a significant radioprotective effect on the brains of rats who undergo high-dose, single-fraction, whole-brain only x-irradiation. To further understand the mechanism by which pentobarbital offers its radioprotective effects, manipulation of GABA binding activity, the primary mode of action of pentobarbital, was studied. Four groups of male Fisher 344 rats received 70 Gy of whole brain radiation in a single dose following one of the i.p. regimens that follows. The first group received pentobarbital (60 mg/kg) and served as the protected group. The second group received ketamine (52.5 mg/kg)/ xylazine (12.5 mg/kg). Both of these compounds have been shown to have no radioprotective effect and this group served as a control group demonstrating baseline radiation toxicity. The third group received picrotoxin (1 mg/kg) and pentobarbital (60 mg/kg) coincidentally. Picrotoxin was administered to evaluate the effect of inhibition of GABA induced synaptic transmission on the radioprotective effect of pentobarbital. The fourth group received bicuculline (6 mg/kg) and pentobarbital (60 mg/kg) coincidentally. Bicuculline was administered to evaluate the effect of inhibition GABA binding on the radioprotective effect of pentobarbital. Survival was followed in each group for 30 days.

The administration of picrotoxin and bicuculline concurrently with pentobarbital inhibit the protective effect seen with the administration of the pentobarbital alone. None of the animals in the picrotoxin group and only 14% of the animals in the bicuculline group survived as opposed to 70% in the group receiving pentobarbital alone. The survival curves of the animals receiving the antagonistic compound were not significantly different than that of the group receiving ketamine, the anesthetic compound known to have no radioprotective effect.

##### Evaluation of the Effect on Pentobarbital Radioprotection by Inhibiting its Ability to Enhance Post-synaptic Conductance Induced by Excitatory Amino Acids

A potential alternative mechanism by which pentobarbital induces its sedative effect is by inhibition of glutamate induced post-synaptic conductance. To evaluate whether another compound with this property would have similar effect, the glutamate antagonist MK801 was utilized prior to the standard 70 Gy whole-brain,

single-fraction dose of x-irradiation. Two groups of Fisher 344 rats were used. Group one received 5.0 mg/kg of MK801 i.p. given following a dose of ketamine (52.5 mg/kg)/ xylazine (12.5 mg/kg). Group two received 2.5 mg/kg of MK801 i.p. given following a dose of ketamine (52.5 mg/kg)/ xylazine (12.5 mg/kg). Survival was followed in each group for 30 days. Neither dose of MK801 rendered any significant radioprotection, suggesting that this mode of pentobarbital activity is not involved in its radioprotective effect. These findings support the hypothesis that the radioprotection seen with pentobarbital is associated with activities which enhance GABA binding and subsequent opening of the chloride ion channel.

### Study of the Radioprotective Effect of Other Barbiturates

As pentobarbital is a radioprotectant, study of alternative barbiturates was initiated to evaluate their potential radioprotective effect. Thirty minutes prior to the standard 70 Gy whole-brain, single-fraction dose of x-irradiation group one received 60 mg/kg of pentobarbital i.p. Group two received 120 mg/kg of phenobarbital i.p. and group three received 180 mg/kg of phenobarbital i.p. A fourth group received 50 mg/kg of thiopental i.p. 15 minutes prior to x-irradiation. In two ancillary studies on thiopental, a similar dose of thiopental was given prior to doses of x-irradiation totaling 50 and 60 Gy, respectively.

Neither phenobarbital or thiopental delivered any radioprotection as measured in the rat model at a dose of 70 Gy. At the lowest tested dose of whole-brain x-irradiation, 50 Gy, thiopental did enhance survival slightly. This data implies that there may be some special significance to the pharmacokinetics of pentobarbital, as compared to other barbiturates, which allows it to have its radioprotective properties.

### Investigation of the radioprotective effect of pentobarbital in a primate model of cerebral radiation toxicity

A concern remains as to the applicability of a model of acute radiation toxicity in a rodent to the radiation toxicity seen in humans with cerebral radiation injury due to treatment of brain tumors. A primate model was designed to better approximate the injury seen in humans which is more chronic in nature. Two groups of animals will be utilized. One will receive 15 Gy of whole-brain, x-irradiation while lightly sedated with ketamine, the anesthetic shown to have no radioprotective effect in the rat model. The second group will undergo radiation after a dose of pentobarbital which results in 30 to 60 second EEG burst suppression. Baseline studies, prior to x-irradiation will include anatomic studies utilizing MRI without and with gadolinium, physiologic studies utilizing positron emission tomography with <sup>18</sup>fluoro-deoxy-glucose (to evaluate neuronal and glial integrity) and <sup>68</sup>Ga-EDTA (to evaluate blood brain barrier and microvascular integrity), and neuroendocrinologic studies utilizing insulin, L-dopa, arginine, thyroid releasing hormone, and gonadotropin releasing hormone as stimulants to measure glucose, cortisol, growth hormone, prolactin, thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone responses. MRI studies will subsequently be done at 1 week, 1 month, 6 months, 12 months, 18 months and 24 months after x-irradiation. Positron emission studies will be done 1 week, 1 month, 1 year and 2 years after x-irradiation. Endocrinologic studies will be carried out 1 week, 1 month, 3 months, 6 months, 12 months, 18 months and 2 years after-irradiation. The animals will be sacrificed two years after x-irradiation and histological exam performed.



The primate study is long term in nature and is ongoing at this time with only a portion of the projected number animals having been enrolled in the study at the time of this report. MRI has not revealed any clearly defined lesions in the animals enrolled in this study as of 1 week and 1 month after x-irradiation. At the 6 month interval study, one animal in the ketamine group has developed one moderate sized lesion in the left hemispheric white matter. No animal in the pentobarbital group has developed a lesion thus far.

On initial review, positron emission tomography has revealed no significant difference from baseline studies developed in any animal.

Endocrinologic studies showed that animals radiated under the influence of ketamine have evidence of early hypothalamic/pituitary functional abnormalities. The glucose response to insulin was magnified, the rebound of glucose levels toward normal was significantly blunted ( $p < 0.02$ ) and remained so through the 6 month interval after x-irradiation with ketamine. No significant alteration occurred in the animals irradiated with pentobarbital. The thyroid stimulating hormone response to thyroid releasing hormone was less marked in the group radiated with ketamine as of 3 months after treatment. Lutienizing hormone response to gonadotropin releasing hormone was significantly decreased by 3 months after x-irradiation in the ketamine group as compared to the pentobarbital group ( $p < 0.01$ ) and remained so after 6 months. Growth hormone response to L-dopa stimulation was transiently decreased to slight degree ( $p = 0.07$ ) in the ketamine group 1 month after irradiation with return to baseline response thereafter. Prolactin response to L-dopa was heightened by 3 months after x-irradiation ( $p < 0.05$ ).

In terms of clinical effects, two of the 3 animals radiated with ketamine for anesthesia succumbed to radiation toxicity as of 6 months following treatment. The third animal remains intact one year following his treatment. Both animals radiated under deep pentobarbital anesthesia remain alive, well and growing 9 months after treatment. The protective effect imparted by pentobarbital as seen in the rat model of cerebral injury is supported in the findings with the primate model thus far. Importantly, there appears to be protection of the hypothalamic/pituitary axis, an area frequently injured in children undergoing radiation, resulting in a significant decrease in the efficacy of this treatment modality.

## 2. Intraarterial Chemotherapy

One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to a drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intracarotid infusion. Our research results have demonstrated the following.

Drug streaming during intracarotid delivery results in maldistribution of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas receive minimal drug. Patients who received intracarotid BCNU were studied with CT scanning, PET scanning, and MRI; the results correlated with histopathology to demonstrate that (1) progressively enlarging cerebral lesions which are often seen on CT and MRI scans following intracarotid chemotherapy may not be tumor recurrence but sites of focal cerebral necrosis; and (2) drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU.

Drug streaming was studied in rhesus monkeys by comparing the distribution of the deposition of  $^{14}\text{C}$ -iodoantipyrine during two methods of intracarotid infusion. A rapid retrograde infusion eliminated the prominent heterogeneous distribution of drug deposition which occurred during the slow infusion (the slow infusion was at a rate analogous to that which is being commonly used clinically). This study indicates that current methods of intraarterial drug delivery, to the brain and other sites, are associated with an unpredictable and variable drug distribution and that this maldistribution can be eliminated by techniques which eliminate intraarterial streaming.

In experiments completed in the DRS by Drs. Shook, Dedrick, Lutz and Doppman, drug streaming was eliminated in an *in vitro* model of the human carotid arterial system by pulsed intraarterial infusion during diastole. This was evaluated by Dr. Stephen Saris in rhesus monkeys with pulsed diastolic injection of labeled  $^{14}\text{C}$ -iodoantipyrine into the internal carotid artery. The pulsed diastolic injection is performed with a prototype pump that is linked to the cardiac cycle so that infusate enters the vessel during the diastolic portion of the flow in the parent vessel. Results using quantitative autoradiography to quantify drug delivery in rhesus monkeys indicated that this technique will allow intraarterial drug delivery with elimination of drug streaming, and will do so at rates of infusion that can be conveniently used clinically.

The presence of streaming, and its elimination by phased, diastolic infusion, has been investigated in 9 glioma patients *in vivo* using positron emission tomography. We demonstrated that standard infusion techniques (continuous infusion of 1-4 cc/min) of  $\text{H}_2^{15}\text{O}$ -labeled water into the cervical segment of the internal carotid artery caused heterogeneous delivery of the infusate to the brain in the distribution of the internal carotid artery. This same infusion technique into the supraclinoid carotid artery produces extremely heterogeneous delivery with large segments of the brain in the ICA distribution receiving either the majority of the infusate, or no infusate. When the cervical and supraclinoid infusions were done with a phased, diastolic infusion, heterogeneous delivery was eliminated. We have now performed 26 intracarotid infusions of cisplatin (70-100 mg/M<sup>2</sup>) without any drug-related complications. These findings confirm our data in other species, and will lead to further clinical studies with intracarotid drug infusions to improve drug delivery, limit toxicity and potentially improve survival of glioma patients.

Since small laboratory animals are often used for pilot experiments in antitumor drug therapy, and in experiments in the areas of pharmacology, physiology and biochemistry, it was important to know if drug streaming occurred after intraarterial infusion into the small arterial systems of these animals. Dr. Stephen Saris, using quantitative radiography, demonstrated that after intracarotid infusions using conventional techniques in the rat, maldistribution of the isotope delivery into the profused brain tissue was a serious problem, and that it could be eliminated by fast retrograde infusion. This observation indicates that if the technique of intraarterial infusion in small laboratory animals is not carefully considered, heterogeneous distribution of the infused agent may lead to invalid or misleading results.

Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high concentration of drug reaches the general circulation, should allow dose escalation

to levels which provide increased tumor response. We have developed techniques which provide regional vascular isolation of a tumor-bearing region by percutaneous catheterization of the afferent and efferent vessels. This permits the venous drainage of the region to be circulated extracorporeally for drug removal before the high concentration of drug reaches the systemic circulation.

A preliminary study, performed *in vitro*, demonstrated that about 90% of the cisplatin in whole blood circulating at 300 ml/min could be removed by hemodialysis using 2 hemodialysis cartridges in series. We then treated 4 patients with cisplatin by intracarotid infusion of a dose that is widely used intravenously. Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion generated tumor exposures 5-15 fold greater than the exposure of the remainder of the body. We then performed 4 treatments in humans using high-dose cisplatin (200 mg/m<sup>2</sup>) combined with drug removal by extracorporeal hemodialysis. The results of the systemic drug levels suggest that the body was exposed to less than 1/2 of the exposure expected if the drug-removal technique had not been used. However, retinal and brain toxicity was intolerable.

### 3. Investigation of Cerebral Edema with Brain Tumors

Brain edema frequently determines the degree of neurologic dysfunction in many disease states. The successful management of vasogenic edema by corticosteroids has had a major impact in the management and quality of survival in patients with brain tumors. The mechanism of action of steroids in the treatment of vasogenic edema is not known; an understanding may optimize the use of steroids in this pathologic state and lead to new treatment methods for various forms of brain edema.

### <sup>68</sup>Gallium-EDTA PET Scanning in Humans with Malignant Brain Tumors

Positron scanning using labelled EDTA is a means to examine brain edema, vessel permeability patterns, and the mechanisms of action of drugs used to treat brain edema. EDTA distributes in the brain extracellular space in a pattern analogous to large water-soluble molecules (e.g., is largely excluded from the normal brain). The PET scan can be employed to indirectly determine permeability in pathologic states by measuring the flux, or clearance (transfer constants  $K_1/K_2$ ) of the labelled EDTA from normal and pathologic areas. In addition, the volume of the extracellular space can be determined by dynamic PET scanning. One of the mechanisms of action of dexamethasone is thought to occur by an effect on the volume of the brain extracellular space. The effects of dexamethasone on the edema/permeability patterns were examined in six patients with metastatic brain tumors using paired PET scans before and after treatment with dexamethasone. The general permeability patterns were further examined in six patients with primary malignant brain tumors. Patients with metastatic tumors had minimal changes in permeability when given high dose dexamethasone, whereas the volume of the extracellular space decreased 15-20% after the dexamethasone.



## Elaboration of a Factor by Malignant Gliomas Which Increases the Permeability of Normal Blood Vessels In Vivo

One of the pathophysiological mechanisms of the production of an intracranial mass effect by primary and secondary malignant tumors is by the tumor eliciting cerebral edema in the surrounding normal tissue. We have demonstrated that media from malignant gliomas in monolayer cultures contains a substance which, when injected intradermally into guinea pigs, increases the accumulation of a circulating radioisotope ( $^{125}\text{I}$ RISA) and a marker dye (Evan's blue) at the site of injection compared to media from fibroblasts, benign brain tumors, normal saline, and tissue culture media. The production of the increased vascular permeability factor in the media from malignant gliomas was abrogated by incubation of the tumor cells with dexamethasone and by inhibition of protein production by cycloheximide. Partial characterization of the factor suggests that it is a 35,000-45,000 molecular weight protein. (See Tumor Biology Unit)

### 4. $^{125}\text{I}$ Iodine Interstitial Brachytherapy for Primary Malignant Brain Tumors. A Phase I-II Study

Interstitial radiation is increasingly being applied to primary malignant brain tumors (MBT) to deliver high local doses of radiation to a disease which is largely a regional problem. Efficacy has been demonstrated, although no consensus has been reached on the total dose, dose rate, treatment volume and isotope characteristics best suited for these tumors. A study was developed in collaboration with the NCI Radiation Oncology Division to address the effects of high-dose interstitial radiation on normal and pathologic tissue using a variety of dose rates and total doses. Patients were divided into three groups with a range of initial dose rates (15, 20 and 30 cGy/hr) and total doses (100-280 Gy) using a homogeneously distributed interstitial approach. Twenty valuable patients with malignant brain tumors (glioblastoma multiforme or anaplastic astrocytoma) were treated with semipermanent  $^{125}\text{I}$  implants using image-directed stereotaxis for placement of multiple source arrays. Patients with recurrent tumors previously treated (5 patients) received only an interstitial boost, while the primary treatment group (15 patients) received whole brain radiation (45 Gy) plus an interstitial boost. Patients did not receive other adjuvant therapy following an implant.

Ten of the twenty patients remain alive. Survival times range from 2-32 months following an implant, and 5-37 months following diagnosis. There were no surgical mortalities; morbidity from surgery arose from 3 complications (one hemiparesis; two local infections). Early radiation toxicity (within 2 months) was limited to radiation-induced peritumoral edema (8 patients) and occurred only in those patients with lesions greater than 4 cm in diameter. Late radiation effects (greater than 2 months) observed by scanning studies demonstrated decreased enhancement of the tumor region, decreased mass effect, atrophic changes in surrounding brain and calcification in areas immediately adjacent to the radiation sources. Fluoro-deoxy-glucose PET studies (six patients) demonstrated focal necrosis corresponding to the treatment volume surrounded by a zone of decreased cerebral metabolism. Karnovsky scores were sustained at the preoperative level in eight patients, fell by 10 points in six patients, and by 20 points in two patients.

Pathologic exam of tissue was performed in seven patients. Three were subjected to surgical procedures: residual tumor was found outside of the implant volume in two patients, and radiation necrosis without tumor occurred in one. Four

patients had autopsies. One had persistent tumor in the implant volume, one had tumor adjacent to the implant volume, two had necrosis only, and one patient had a contralateral tumor which developed 16 months after diagnosis.

The conclusions of the current study are (1) the interstitial approach developed has acceptable surgical and radiation risks with respect to early toxicity, and (2) the use of multiple arrays of low activity sources to distribute the radiation dose has a lower incidence of symptomatic radiation necrosis, (3) smaller treatment volumes have fewer toxic reactions regardless of the dose rate and total dose, and (4) no conclusions can yet be reached regarding the best dose rate and total dose.

## 5. Immunotherapy of Brain Tumors

Investigation of the efficacy and toxicity of adoptive immunotherapy using interleukin-2 (IL-2) and lymphokine activated killer cells (LAK) for treatment of brain tumors, including investigation of the antitumor effect of these therapies in vitro in brain tumor models in laboratory animals and in the clinic.

### The Effect of IL-2 on the Blood-brain Barrier in the 9L Gliosarcoma Rat Model

When used to treat extracranial cancers, the major dose-limiting toxicity of IL-2 is a generalized increase in vascular permeability. We have observed a high incidence of treatment-induced cerebral edema in brain tumor patients who received intravenous or local IL-2 therapy. To investigate the effect of IL-2 on the normal cerebral microvasculature, and on the vasculature of brain tumors, we determined the change in vascular permeability after IL-2 therapy in the rodent 9L gliosarcoma model. <sup>14</sup>Carbon-aminoisobutyric acid was used to determine local blood-to-tissue transfer constants (K) in 22 Fischer rats with intracerebral 9L gliosarcomas that received either high dose parenteral IL-2 or a control injection. In tumor and peritumoral tissue, the transfer constants in IL-2 treated animals [ $89.6 \pm 14.6$  (mean  $\pm$  S.E.) and  $35.8 \pm 6.0$ ] were larger ( $p < 0.05$ ) than in control animals ( $61.4 \pm 6.4$  and  $14.6 \pm 2.2$ ). In contrast, in normal frontal and occipital tissue contralateral to the tumor-bearing hemisphere, there was no difference between the transfer constants in IL-2 treated and control animals. Furthermore, treatment of animals with IL-2 excipient caused no change in permeability as compared to animals treated with Hanks balanced salt solution.

This study demonstrated that parenteral injection of IL-2 increase BBB disruption in tumor-bearing rat brain, but does not increase the vascular permeability of normal brain. Methods to prevent this increased tumor vessel permeability are required before parenteral IL-2 will be used safely for the treatment of primary or metastatic brain tumors.

### Adoptive Immunotherapy of Intracerebral Metastases in Mice

Lymphokine activated killer (LAK) cells nonspecifically destroy neoplastic cells, but not normal cells. Parenteral treatment with interleukin-2 (IL-2) alone, or IL-2 and LAK cells, reduces tumor load and prolongs survival in mice with pulmonary, peritoneal, or hepatic metastases. To compare the efficacy of this adoptive immunotherapy against intracerebral and pulmonary metastases, we performed intracardiac and intravenous (IV) injections of  $10^5$  KHT sarcoma cells in C3H mice to



create brain and lung metastases, respectively. The mice were treated with either parenteral IL-2 (7500 units three times daily [ttd] on days 3-7 after tumor injection), IL-2 + LAK (7500 units tid on days 3-7, and 10<sup>8</sup> LAK cells IV on days 3 and 6 after tumor injection), of IL-2 excipient (tid on days 3-7 after tumor injection). As compared to control animals, pulmonary metastases on day 14 after tumor injection were reduced or eliminated in animals treated with both IL-2 and IL-2 + LAK ( $p < 0.01$ ). However, in the same animals there was no reduction in the number of brain metastases.

We conclude that IL-2 and IL-2 + LAK therapy is highly effective against lung metastases, but ineffective against brain metastases in this tumor model. Furthermore, this modification of a previously existing model of murine brain metastasis provides a method for concurrently evaluating the effectiveness of treatments for intra- and extra-cranial cancers.

### Immunotherapy of Gliomas in Rats

Laboratory efforts were initiated to examine the immunology of brain tumors to determine whether immunotherapy techniques that take advantage of tumor infiltrating lymphocytes might have a role in treating glial tumors. IL-2 was administered to rats bearing syngeneic gliosarcomas for five days TID while control animals received equal injections of balanced saline solution. Microscopic examination of the brains of animals from both groups studied at three, seven, ten and 14 days after starting treatment demonstrated no lymphocyte infiltration in tumors. Therefore, the small increase survival seen in brain tumor-bearing rats treated with systemic IL-2 (20% increase) is not related to increased lymphocyte infiltration. This data suggests that the lack of CNS tumor response seen with systemic LAK cell and IL-2 treatments of patients with primary glial and metastatic tumors may be due to the inability of LAK cells to respond to tumor across the blood-brain barrier. It also suggests that the yield of tumor infiltrating lymphocytes from the intracranial tumors will be small.

### Development of Optimal Technique for Placement of LAK Cells Directly into Brain and Brain Tumors

Culture of density gradient-separated leukocytes in recombinant IL-2 results in the differentiation of rat splenic leukocytes into tumoricidal LAK. We first optimized the conditions for generation of LAK with respect to IL-2 dosage and time course and the parameters for storage of LAK under liquid nitrogen in preparation for use. Robert Plunkett, M.D. then demonstrated that intracranial administration of chromium-51 labelled LAK cells was optimal at  $2 \times 10^7$  cells/ml in 10 microliters at 5 microliter per minute via 23 gauge Hamilton syringe needles. Recovery of lymphocyte associated radioactivity from brain after dissociation was nearly 50 percent (see CNS Implantation Unit). These results established the optimal parameters and conditions to evaluate IL-2 and LAK immunotherapy in the treatment of malignant gliomas.

The success of intratumoral immunotherapy, as is being investigated in brain tumors at several centers, depends on the maintenance of peak LAK cell cytotoxicity. We tested the influences of cytotoxicity of activated LAK cells recultured with prolonged exposure to various concentrations of IL-2 and dexamethasone. Cytotoxicity was maintained in LAK cells recultured for three days and five days with

1000 units/ml of IL-2, but was reduced by 50% at three, and by 90% after five days, without IL-2. Reculturing LAK cells incubated with dexamethasone demonstrated a 10-30% decrease in cytotoxicity. This demonstrated the need for prolonged administration of IL-2 to assure peak LAK cell cytotoxicity. It also suggests that inhibition of the effectiveness of LAK cell and IL-2 therapy with dexamethasone treatment will limit the use of steroids against cerebral edema during adoptive immunotherapy with LAK-IL2.

### Clinical Studies of Adoptive Immunotherapy

- a. We studied the clinical and neuroradiological effects in 10 patients treated with parenteral administration of repeated doses of IL-2 to evaluate neurological toxicity prior to Phase II trials in patients with brain tumors. Three patients had malignant gliomas, and six patients had extracranial cancer without evidence of intracranial metastasis. All were treated with intravenous doses of  $10^5$  units/kg tid for 5 days. The patients with gliomas received cranial computerized axial tomographic (CT) scans before initiating IL-2 therapy, and during later stages of treatment. The patients with extracranial cancer received pretreatment and late treatment T-2 weighted magnetic resonance imaging (MRI).

After two to eleven doses of IL-2, the patients with gliomas had marked neurological deterioration that was associated with a mild to marked increase in peritumoral edema and mass effect on CT scan. With cessation of treatment, and appropriate supportive care, all returned to their pretreatment state. The patients with extracranial cancer were either neurologically unchanged, or underwent minor, transient changes (lethargy, confusion) in mental status. The signal intensity of MRI images was quantified and compared in eight anatomic regions of interest. In six of seven patients, there were increases in gray and white matter signal intensity consistent with increased cerebral water content. The mean changes (percentage  $\pm$  standard error) were 12.6%  $\pm$  7.3% in the gray matter and 17.0%  $\pm$  6.2% in the white matter.

This study demonstrated that treatment with high dose parenteral IL-2 is not tolerated by patients with gliomas due to increase cerebral edema. In patients with extracranial cancer, but no brain disease, parenteral IL-2 induces an increase in the cerebral water content of both gray and white matter.

- b. Treatment was tested using regional delivery of IL-2 and LAK cells directly to the site of malignant primary brain tumors. These protocols investigated the clinical response of the infusion of repeated doses of LAK cells and IL-2 in patients with cystic and solid intrinsic malignant primary CNS tumors. Nine patients were treated with 15 courses of immunotherapy. One of the nine patients had a partial tumor response, as measured by contrast enhancing CT scans following therapy. All patients developed increased brain edema in surrounding the tumor and experienced some increase in focal neurologic dysfunction. Five patients suffered permanent worsening of their neurologic status.

Tumor cyst fluid was obtained during immunotherapy and tested for IL-2 and cell numbers. IL-2 was not detected in the tumor cyst prior to treatment, but was present after infusion ( $100$ - $1,000$  IL-2 units/ml) and decreased with time (an intra-tumor cyst half-life of about 8 hours). These studies suggest that there is

a limited *in vivo* response of primary CNS tumors to this treatment and that the treatment is associated with significant patient morbidity. For the above reasons these treatment protocols have been closed.

## 6. Studies of Patients with von Hippel-Lindau Disease

Von Hippel-Lindau's disease is an inherited disorder in which patients suffer from hemangioblastomas of the retina, cerebellum and spinal cord as well as renal cell carcinomas, pheochromocytomas and visceral cysts. The disease is passed in an autosomal dominant pattern which has been linked by recombinant fragment length polymorphism analysis to the short arm of the third chromosome. The defective gene is in the 3p 14 region.

In collaboration with Drs. Berton Zbar and Marston Linehan of the NCI and Dr. Michelle Filling-Katz of NINDS, we studied 13 separate tumors from 5 patients, analyzing them for loss of alleles on the third chromosome. These tumors demonstrate loss of one copy of the vHL allele. But in the tumor cells, the loss is of the normal, wild type allele inherited from the normal parent. This loss of the balancing wild type allele leaves the tumor with only the abnormal allele inherited from the parent carrying von Hippel-Lindau disease.

This supports the theory that the tumors in vHL disease are caused by the loss of a tumor suppressor gene similar to that seen with retinoblastoma and type II neurofibromatosis; the affected parent contributes a defective copy of the gene allowing cells at risk to develop tumors when the other copy, the balancing wild-type gene from the normal parent, is lost or rendered nonfunctional.

The cell of origin of cerebellar hemangioblastomas remains unproven. We, in collaboration with Dr. David Katz of neuropathology, are studying these tumors using light and electron microscopy. Immunohistochemical stains specific for neural, glial, neuroendocrine, vascular, and other mesenchymal tissues are being used to determine the cell of origin of the hemangioblastomas. Electron microscope is being used to investigate reports of 'neurosecretory granules' in these tumors. Tumor cell lines are also being sustained in culture to study the cells of origin and the growth characteristics of these cells.

Clinical studies are also being undertaken with these patients. The spinal and cerebellar tumors permit comparisons of the sensitivity and precision of current diagnostic and imaging techniques used to study these patients, thus allowing a better understanding of how to care for patients with this disorder.

We have determined that syrinxes of the spinal cord in this condition resolve spontaneously following tumor removal alone, and that the tumor-associated syrinx does not require separate treatment.

Surgery on these patients has also revealed that the spinal tumors are almost exclusively associated with the dorsal spinal roots or found on the dorsal aspect of the cord and can frequently be found exclusively in the extra-medullary intra-dural space (outside the spinal cord); these observations have previously not been reported in the literature concerning the surgical treatment of these lesions. They also provide insight into the cell of origin of these tumors.



## B. Pituitary Tumors

### 1. Venous Sampling to Establish the Diagnosis and Location of Hormone-Secreting Pituitary Microadenomas

We have now performed bilateral and simultaneous inferior petrosal sinus sampling in over 200 patients with Cushing's syndrome. The results are used (1) to confirm the diagnosis of Cushing's disease preoperatively and, (2) to determine the half of the pituitary gland in which a microadenoma resides. The study has been particularly rewarding and has demonstrated the following: (1) sampling from both inferior petrosal sinuses simultaneously and consistently distinguishes patients with Cushing's disease from those with ectopic ACTH syndrome (greater than 99% accuracy); (2) sampling from a single inferior petrosal sinus, as has previously been general practice, to establish the diagnosis of Cushing's syndrome, may be misleading and could result in an incorrect assumption of the source of excess ACTH secretion in as many as 43% of patients with Cushing's syndrome; and (3) preoperative sampling for ACTH concentrations in the inferior petrosal sinuses determines the site of ACTH-secreting microadenomas within the pituitary gland with about 85% accuracy. Therefore, bilateral sampling permits the surgeons search for small microadenomas to be focused to one side of the gland, which should be helpful in finding smaller tumors. If no tumor is found, the half of the gland containing the microadenoma can be removed. We have had only two treatment failures in over 60 previously untreated patients with Cushing's disease who have undergone preoperative sampling. This technique is now being widely employed in the evaluation of patients with Cushing's syndrome.

We investigated the use of additional pituitary hormones (prolactin, TSH, and alpha subunit of HCG), which are presumably secreted symmetrically into the cavernous sinuses and inferior petrosal sinuses by each half of the pituitary gland, to see if they could be used to standardize ACTH measurements against unequal dilution of the venous blood in the two inferior petrosal sinuses for tumor localization. None of these hormones proved helpful as a means of measuring unequal dilution of venous blood between the pituitary gland and the inferior petrosal sinuses. However, these hormones, particularly prolactin were consistently elevated in the blood from the inferior petrosal sinus on the same side of the tumor. This suggests that the hormonal secretion of pituitary microadenomas influences the hormonal secretion of the adjacent, normal, pituitary tissue and supports the possibility of functional paracrine regulation of hormonal secretion in the anterior lobe of the pituitary gland.

### 2. Radiologic Imaging and Cushing's Syndrome

To evaluate the CT scan as a means of aiding the differential diagnosis of patients with Cushing's syndrome and as a mechanism for tumor localization pre-operatively, we prospectively evaluated the results of CT scanning in 57 patients with Cushing's syndrome. All 8 patients with ectopic ACTH secretion had normal CT scans, all 8 patients with pituitary microadenomas had focal pituitary abnormalities that were visible on the CT scan, but only 7 of the 42 patients with pituitary microadenomas and Cushing's disease had demonstrable abnormalities on CT scanning. The conclusion of this study is that CT scanning is only occasionally helpful in locating, pre-operatively, the site of an ACTH-secreting pituitary microadenoma, and is therefore only rarely beneficial as a diagnostic aid in the differential diagnosis of patients with Cushing's syndrome.

MRI scanning was used with and without the new paramagnetic contrast agent, gadolinium-EDTA, to evaluate patients with Cushing's disease preoperatively. This technique permitted identification of the adenoma in about 50% of those patients with surgically-proven microadenomas who have negative CT-scans. Therefore, CT and MRI with Gd-EDTA identified adenomas preoperatively in about 65% of the patients. It was learned that the timing of the MRI after administration of Gd-EDTA was critical in optimal use of the technique, as sequential imaging demonstrated scans taken  $\leq 5$  min after Gd-EDTA injection to be most sensitive.

### 3. Repeat Pituitary Surgery in Patients with Cushing's Disease Despite Previous Treatment

The ideal treatment of patients with Cushing's disease should eradicate tumor, reverse hypercortisolism, and avoid the requirement of longterm endocrine deficiency and permanent hormonal replacement therapy. These goals of therapy are only met with selective removal of the offending pituitary adenoma. However, patients who have received prior pituitary treatment, but still have hypercortisolism, are usually not considered candidates for repeat surgery. We investigated the role of repeat transsphenoidal surgery in 29 consecutive patients with prior pituitary treatment and persistent, or recurrent, Cushing's disease. In 20 of the 29 patients an endocrine cure was achieved by repeat transsphenoidal surgery. Our experience demonstrates that in patients with prior pituitary treatment, if an adenoma can be identified intraoperatively and selectively excised, the patient can be expected to have resolution of hypercortisolism and preservation of normal pituitary function. They further indicate that if a distinct adenoma cannot be identified intraoperatively, the chances of effecting resolution of hypercortisolism and preserving pituitary function are quite low.



### C. Surgical Treatment of Medically Intractable Epilepsy

The aim of the surgical arm of the NINDS epilepsy program is to develop surgical techniques that allow more accurate localization and safest resection of epileptogenic foci than can be achieved with methods now available. The development and implementation of surgical treatments for patients whose seizures are intractable to currently available medical and surgical therapies is the immediate and long-term goal of this program.

Special subdural surface electrodes designed and built at NIH in collaboration with the BEI Branch of DRS are now being implanted in selected patients so that EEG recordings can be obtained directly from the cortical surface for much longer periods than is possible during intraoperative electrocorticography. During the period of implantation these electrodes are also utilized for focal cortical stimulation to discriminate areas that can be safely resected from areas critical for motor, sensory, language, memory related and other functions. Such discrimination is crucial in the topographic identification of overlap between critical cortical areas and epileptic foci during surgical procedures. During the next several years a subset of patients with epileptogenic foci originating in the language dominate hemisphere will undergo implantation of a new type of subdural electrode designed and built here at NIH. Recordings and functional stimulation mapping utilizing these electrodes during a two to six day period preceding resective surgery should allow a maximum and safe surgical resection to be performed under general anesthesia in these patients who are difficult or impossible to cure using current approaches.

Analysis of the data from several recently developed methods for localizing epileptic foci is being performed to determine comparative sensitivity and reliability of the various techniques. During surgery for focal epilepsy, depth and special subdural (surface cortical) electrodes are being used to record from deep structures inaccessible by routine electrocorticography to identify and confirm areas of potential epileptogenic activity suggested by the investigational preoperative methods of PET scanning, magnetoencephalography, MRI and magnetic resonance spectroscopy (MRS).

MEG offers the possibility for localization of abnormal seizure-related electromagnetic phenomena in 3-dimensions. NO other methodology currently available can noninvasively acquire this type of information, which is highly desirable for identifying epileptogenic foci for surgical excision and in the study of basic mechanisms in epilepsy. In several cases performed here at NIH we were able to predict correctly the localization of foci causing complex partial seizures using MEG data in patients whose preoperative EEG (in retrospect) gave misleading and/or false localizing information. These foci were successfully extirpated and would have been missed if a standard temporal lobectomy had been performed. The new 7 channel MEG, now in place, promises even greater 3-dimensional localizing capability, and we are now studying focal motor seizures as well as complex partial and generalized tonic-clonic seizures with this technique.

In selected patients we are now implanting subdural electrodes modified so that in addition to their recording capability, they can be utilized to create a current dipole. This dipole can then be detected by the technique of MEG allowing, for the first time, direct proof of the ability of the MEG to accurately and previously predict the location of a current dipole source within the three dimensional space of the human cranium. Patients requiring chronic subdural recordings from different

geometric sites to localize the seizure focus participate in this MEG study, so that we can gain greater confidence in the ability of MEG to localize a current dipole source within the head of a patient to an accuracy calculated in our first experiments, within 5 mm of the true dipole source.

Electrocorticography under general anesthesia at the time of resective surgery often generates data of uncertain significance. An ongoing study is carefully evaluating the effect of different anesthetic agents on interictal spikes, background Data gathered from the first group of patients studied with this protocol reveal that isoflurane suppresses the number of abnormal spikes during surgical electrocorticography when compared to recordings immediately before and after its use in the same patient. Enflurane under similar conditions is seen to increase the number of abnormal spikes and to produce paroxysms of synchronous high-voltage spikes. Data reported to date suggests that isoflurane can suppress epileptogenic tissue and that both isoflurane and enflurane can distort the ECoG, confounding accurate identification of the seizure focus during surgery. When used judiciously, however, enflurane may be a potent synchronizer and activator of the epileptogenic focus, permitting easier identification.

As abnormal foci, which appear to cause clinical seizures, are better defined an attempt is being made to identify chemical derangements in these focal areas. One approach includes the measurement of neuropeptides and transmitter substances in fresh, frozen tissue samples of epileptogenic cortex, removed during surgery. This is being done in collaboration with Dr. Suzanne Nadi, MNB.

Another approach that should yield information about the chemical changes that are thought to occur in tissues in which seizures arise is magnetic resonance spectroscopy (*in vivo* NMR spectroscopy). Under a new protocol, pH and lactate measurements are being made from specific regions of interest in seizure patients and are compared with measurements from similar regions in the opposite hemisphere and with measurements carried out in normal volunteers. This study is being carried out in collaboration with Dr. Giovanni DiChiro and Dr. Jeffery Alger, NIS.

One of the most severe postoperative deficits suffered by patients with temporal lobectomy is unpredictable recent memory loss. It is assumed that this is the side opposite the resection. Routine preoperative Amytal (WADA) testing has been only minimally successful in predicting post-operative memory deficits in patients who receive unilateral hippocampectomy for seizures. There, a study of (super selective) posterior cerebral artery Amytal testing will begin in collaboration with Dr. John Doppman of the Radiology Department, CC which should selectively block hippocampal function unilaterally in the awake patient leading to predictions about the state of postoperative memory function after this tissue is removed.

## D. Vascular Disorders of the CNS

### Spinal Arteriovenous Malformations

#### Arteriographic Findings

A retrospective review of the clinical features, arteriographic findings, and treatment of 81 patients with spinal arteriovenous malformations demonstrated that there are distinguishing clinical features in patients with arteriovenous malformations of the spinal cord compared to those of patients with arteriovenous fistulas of the spinal dura. They also suggested that the demographic characteristics of spinal arteriovenous fistulas suggest that this is an acquired and not congenital lesion and support true spinal arteriovenous malformations of the spinal cord as congenital lesions. The findings indicate that true arteriovenous malformations of the spinal cord produce myelopathy as a result of the high flow through these lesions with its associated arterial steal phenomenon, and the multiple hemorrhages experienced by these patients with spinal dural AV fistulas develop myelopathy as a result of increased pressure of the venous system of the spinal cord. The results also demonstrate that the response to treatment is better with patients with spinal dural AV fistulas and, if the patient's fistula can be identified early in the course of the illness, that most neurological function of the spinal cord can be preserved by treatment.

#### Magnetic Resonance Imaging in Patients with Spinal Arteriovenous Malformations

Magnetic resonance imaging, used prospectively to evaluate patients with spinal arteriovenous malformations, demonstrated the presence and the site of arteriovenous malformations of the spinal cord. However, it failed to demonstrate the site of, and only occasionally demonstrated the presence of, spinal dural AV fistulas. In patients with arteriovenous malformations of the spinal cord it permitted sequential assessment of the success of therapy to obliterate the malformations. Therefore, it proved to be a valuable and noninvasive technique to investigate patients suspected of having spinal arteriovenous malformations and to sequentially follow the course of these lesions during and after therapy.

#### Cranial Dural AV Fistulas Producing Myelopathy

In three patients, referred for treatment of spinal AVMs, cranial dural AV fistulas with venous drainage from the fistula limited to the spinal venous system were identified. Interruption of the venous drainage of the fistula induced recovery of cord function and obliterated the fistula in all 3 patients. Cord edema, demonstrated by MRI, which occurred as a result of venous congestion, was reversed. These observations indicate that (1) venous congestion alone can cause myelopathy, (2) patients who appear to have spinal AVMs but who have negative spinal arteriography should have carotid arteriography, and (3) simple interruption of the vessel which drains a cranial dural AV fistula intradurally is sufficient treatment for obliteration of the AV fistula (surgical excision is not required).

### Foix-Alajouanine Syndrome and Spinal AVMs

The clinical course and response to treatment of 5 patients with Foix-Alajouanine syndrome demonstrated that in many patients the early phase of myelopathy is due to venous congestion, and not venous thrombosis. Therefore, prompt treatment induces partial or complete reversal of myelopathy in these patients, and it is not necessarily an irreversible syndrome, as it has often been considered.

### Embolic Occlusion of Spinal AVMs

Five of six patients with spinal AVMs were shown to have recanalization of the AVM and relapse of myelopathy within one year of embolic occlusion of the AVM. Embolic occlusion of spinal AVMs, as is known to occur with cerebral AVMs, was thus shown to be only temporarily effective.



## II. BIOCHEMISTRY SECTION

Richard J. Youle, Ph.D. - Chief

### A. Monoclonal Antibody Mediated Killing of Tumor Cells

The Section of Biochemistry, headed by Dr. Richard Youle, is studying the use of monoclonal antibodies to kill disease causing cells. Monoclonal antibodies which selectively bind tumor cells can be generated, but alone are usually not cytotoxic to the tumor. Toxic proteins such as ricin and diphtheria toxin can be chemically linked to monoclonal antibodies. The new hybrid molecules bind tumor cells via the antibody moiety and then kill the cells via the toxin moiety. The toxins used are enzymes that catalytically inactivate protein synthesis in target cells with only one or two molecules in the cytoplasm killing a cell. However, the non-target cell toxicity of the toxins must be blocked with excess lactose to prevent toxin binding and this currently limits this approach to in vitro applications. The cell-type-specific reagents have immediate clinical application in vitro in bone marrow transplantation where T cell depletion improves allogeneic transplantation. The Section of Biochemistry is collaborating in clinical trials in bone marrow transplantation at the University of Minnesota. Twenty four patients have now been treated with immunotoxin purged bone marrow as the sole prophylaxis for graft-versus-host disease. Comparing the outcomes with historic controls treated post-transplant with methotrexate, several conclusions can be drawn. The patients had a milder course as evidenced by a significantly shorter hospitalization. Engraftment of donor marrow occurred with a shorter time until leucocyte generation and no severe graft-versus-host disease was seen. Clinical trials of immunotoxin treatment of bone marrow are continuing to increase the patient population and thus the statistical significance of the apparent benefits.

The major goal of the laboratory is to develop immunotoxins which will selectively kill tumor cells in vivo. Currently the limiting steps for antibody-toxin hybrids are (1) the entry of the toxin molecule into the cell; and (2) in vivo access of the drug to the tumor cells.

To promote access of monoclonal antibody-toxin conjugates to tumors we have focused on tumors localized in body compartments such as the brain and the peritoneal cavity. The brain may be an optimal compartment for antibody modulation of cell function.

The discovery of point mutants of DT that increase immunotoxin selectivity in FY 88 and the demonstration of immunotoxin activity in animal models of leptomeningeal cancer in FY88 have been moved toward clinical trials in FY 89. Extensive toxicity testing has shown that a 100-1000 fold therapeutic window exists between the concentration toxic to tumor cells and the dose tolerable in rats, guinea pigs and monkeys. This promising new drug will soon be tested clinically and may offer a new form of therapy. We have submitted a protocol to the NINDS IRB and met with the FDA.

To improve entry of immunotoxins into cells we have modified toxins chemically, molecular biologically and with monoclonal antibodies. We are studying the structure-function relations of the toxin molecule and the cell biology of toxin internalization and membrane penetration.



Toxins may be best adapted for tumor specific toxicity by alterations of amino acid sequence at the gene level. To begin improving immunotoxins at the gene level we have worked with the prokaryotic toxin, diphtheria toxin. Intact diphtheria toxin was linked to a monoclonal antibody specific for human T cells and was found to specifically kill target cells at  $10^{-12}$ M. The rate of specific killing was 10-fold faster than previously reported immunotoxins. This model system was then used to study cloned fragments of diphtheria toxin. In collaboration with Dr. Larry Greenfield, who has cloned diphtheria toxin (DT), we deleted the C-terminal 15kD region shown to include the cell surface binding site. This left the toxin A subunit plus a 17 kD fragment of DT B chain thought to facilitate transmembrane transport. When linked to monoclonal antibodies, this truncated DT was 100-fold more toxic than DTA chain linked to antibody, and the toxicity was blocked by excess antibody. The cloned DT fragment was 1000 times less toxic to guinea pigs than the native toxin. Therefore, the fragment of DT B chain included by cloning increased target cell toxicity more than non-target cell toxicity indicating that separation of B chain entry functions from binding was accomplished to some degree. We compared intact DT linked to monoclonal antibody with the cloned fragment of DT, which showed the C-terminal fragment of DT further increased the antibody mediated toxicity 100-fold. We are currently studying conjugates of 3 point mutants of diphtheria toxin linked to antibodies in attempts to include the 100-fold activation by the C terminus of DT while omitting regions causing non-target cell killing. Recent results show that point mutations can increase selective cytotoxicity up to 10,000-fold.

We are studying the mechanism of toxin entry into cells to allow improved design of tumor-specific reagents. Toxins like ricin and diphtheria toxin bind the cell surface, are endocytosed, then cross the membrane surrounding the endocytotic vesicles to reach the cytosol where they inactivate protein synthesis. The rate limiting step in toxin and immunotoxin activity is a transport across the membrane to the cytosol. How and where this transport step occurs is unknown. To investigate this question we have used hybridoma cells which secrete monoclonal antibodies that block ricin toxicity. We have found that these cells are themselves resistant to ricin because of the antibody they synthesize. We found that the resistance was not caused by extracellular or cell surface antibody but by antibody within the cell in route to secretion. This means that ricin must pass through the protein secretory pathway, comprising the endoplasmic reticulum, golgi apparatus, and secretory vesicles, before entering the cytosol. This new approach may be applied to study other toxins, hormones and macromolecules which enter cells by receptor mediated endocytosis.

We have recently identified point mutations in a hydrophobic domain of diphtheria toxin B chain that inhibit membrane translocation activity 90%. This results shows a role of protein 309 in the translocation activity of diphtheria toxin.

We have found ways to block the immune response animals generate against immunotoxins. Injection of a monoclonal antibody against the helper T cell CD4 antigen completely prevent the primary immune response and 90% to prevent the secondary immune response against immunotoxins.

In an effort to develop immunotoxins to treat AIDS we have collaborated with Biogen to create CD4-toxin conjugates that specifically kill HIV infected cells. This project has just begun and the bioactivity of these new toxins is not yet known.

## B. Programmed Cell Death in the Nervous and Immune System

Our laboratory has begun a project to study the mechanism and physiologic role of programmed cell death. In both the nervous system and the immune system, massive numbers of cells die during normal development. In the spinal cord, for example, over half the neurons die during fetal development. The immune system also has large cell loss at precise stages in development such as thymocyte death and thymus involution at puberty. The physiologic role and the biochemical mechanism of programmed cell deaths is unknown. Understanding the mechanism and regulation of such physiological cell deaths may shed light on neurodegenerative diseases and immunodeficiency disorders.

In our initial studies we found that thymocytes could be induced to die by the same signal that stimulates mature T cells to proliferate. RNA and protein synthesis inhibitors block new gene expression and block the programmed cell death. In addition, we found that prolactin can block the glucocorticoid induced cell death of thymocytes. This result has significance for clinical immunosuppression and autoimmune disorders.

We are extending our studies into the nervous system by examining the mechanism of thymocyte death in various atoxic mutant mice and by examining the mechanism of glutamate induced neuron death in vitro and in vivo. We have found that the morphology of thymocyte programmed cell death by Normarski optics mimics the morphology of neuronal programmed cell death.

In conjunction with our studies on immunotoxins we have made now animal models of purkinje cell death. Injection of immunotoxins can lead to 70-80% loss of purkinje cells without affecting other neurons. This discovery not only permits generation of a new animal model but also points toward the possible mechanism of purkinje cell loss in a variety of neurological disorders. Purkinje cell loss in inherited metabolic diseases may be a direct consequence of the high endocytosis rate expressed by purkinje cells.

### III. Central Nervous System Implantation Unit

Robert J. Plunkett, M.D., Head

#### A. Tissue Implantation in Parkinsonian Animal Models

The effect of tissue implants into the caudate nucleus of parkinsonian animals is being studied from behavioral, biochemical, and histological viewpoints. The models utilized are the hemiparkinsonian monkey and the the hemiparkinsonian rat model developed in our lab. Our previous work showed that grafts of fetal mesencephalon lead to recovery from the motor deficits in the monkeys, while adrenal allografts were less efficacious. We have now performed adrenal autografts and sham cavitation of the caudate and followed these animals for up to one year. In both groups, the improvement seen was modest and occurred over many months, unlike the fetal grafts which lead to improvement within weeks.

We are doing further studies of the MPTP-parkinsonian monkeys and the effects of dopaminergic agonist therapy. This involves receptor autoradiography to assess up or down regulation after specific agonist/antagonist therapy. Glucose utilization (2-DG) is being used to evaluate regional metabolism in the parkinsonian monkeys after acute or chronic dopaminergic therapy. Finally, we are performing cell counts in the midbrain of hemiparkinsonian monkeys to determine the exact numbers of nigral and tegmental dopaminergic neurons left after MPTP treatment.

One phenomenon which we have observed to varying degrees after all implants and after cavitation alone is sprouting of intact host dopaminergic fibers, presumably from the intact meso-limbic system. Efforts are currently underway to trace the sprouted fibers using retrograde and anterograde tracers.

To further investigate this sprouting, we implanted fetal monkey amnion into hemiparkinsonian monkeys and observed significant behavioral improvement (almost as good as seen after fetal dopaminergic implants). We are studying these animals with a variety of techniques including 6-fluorodopa PET, receptor autoradiography, and immunohistochemistry. In addition, the gelatin sponge placed at the time of cavitation, as well as samples of CSF from these animals are being studied for their ability to promote neurite outgrowth (see below). We have also developed a new *in vivo* microdialysis system to study biochemical changes in the implanted monkeys. The system appears useful to compare the normal and hemiparkinsonian sides and to detect increased dopaminergic activity after implantations.

Since many of the cells or tissues which may be used for implantation are allografts, we have established the appropriate immunohistochemical techniques to characterize the immune or inflammatory response to grafting. Using these techniques, have determined that rejection did occur in one of the animals receiving a fetal dopaminergic implant. In addition, we are characterizing the MHC status of the various cells and tissues we are implanting. We have performed two experiments in which an allograft or a xenograft was placed in monkeys, and peripheral blood examined for the IL-2 receptor and the lymphocytic response. The goal is to see if rejection can be recognized by the peripheral response.

## Neurite-promoting Activity from Damaged Central Nervous System and other Tissues

We have previously established that there is neurite-promoting activity at the site of tissue injury in the cortex of rats or monkeys, and in the caudate of monkeys. The bioassay used is qualitative, with NGF used as the standard. We are trying to quantify the assay using neurofilament protein as a marker. In addition, two other assays are being developed: the rat superior cervical ganglion and the fetal mesencephalon of rat. These are closer to the *in vivo* situation in parkinsonian models. We have studied several other rat, monkey, and human tissues using these assays and have found significant neurite-promoting activity in fetal kidney and amnion and less activity in term amnion. Other cell lines and tissues are under study.

## Cellular Implantation in a New Rat Model of Parkinsonian

In order to have a small animal model to perform implantation experiments, we modified the standard hemiparkinsonian rat model. The changes we made allow destruction of the substantia nigra while sparing the ventral tegmental area (this model is analogous to the MPTP-hemiparkinsonian monkey model). In this model there is some tyrosine hydroxylase activity in the medial caudate, but very consistent and stable turning in response to amphetamine. We chose to examine cellular implants delivered stereotactically to see if they would influence the turning behavior.

The first study consisted of three groups of hemiparkinsonian rats: non-implanted, sham-implanted, and rats receiving implants of peritoneal inflammatory cells (predominantly macrophages and T-cells). The control and sham animals did not change their rotational behavior over 8 weeks, as in the leukocyte implanted animals. Immunohistochemistry with antibody to tyrosine hydroxylase showed increased TH reactivity in the implanted animals. Dopamine determination by HPLC showed a trend towards increased dopamine in the implanted group. We are now doing implants of pure macrophages and T-cells to determine if the response can be seen with either cell alone.

## Characterization of the development of the Fetal Mesencephalon

We have studied the ventral mesencephalon in rodents and in primates and established the pattern of differentiation of dopaminergic neurons. The expression of the enzyme tyrosine hydroxylase (TH) was used as a marker of dopaminergic activity. Human ventral mesencephalon from 6-12 gestational week fetuses can be successfully placed in culture; there is glial proliferation but no neuronal expansion. There are neurons at this developmental stage which stain positively for TH and extend neurites. We are currently examining fetal tissue from ectopic pregnancies to determine if there are viable dopaminergic neurons in this situation.

## Fetal Pituitary and Pituitary/Hypothalamic Transplants

We have developed a successful model of transplantation of fetal rat pituitary + hypothalamus into hypophysectomized rats. We use small tissue fragments delivered stereotactically to the region of the median eminence. There are peripheral plasma levels of some anterior pituitary hormones post-transplantation, and immunohistochemical evidence of surviving anterior pituitary cells. There is



some cellular infiltrate in the implants; we are currently characterizing these cells using specific monoclonal antibodies. We have started preliminary work using monkey fetal pituitary tissue. In addition, we are doing in vitro work with cell lines transfected with the growth hormone gene.



#### IV. Tumor Biology Unit

Marsha J. Merrill, Ph.D., Head

##### A. Vascular Permeability Factor Produced by Glioma Cells

Cerebral edema is a significant cause of the neurological deficits and elevated intracranial pressure associated with malignant brain tumors, and is an important challenge in the clinical management of patients with this disease. We have determined that medium conditioned by cultured human glioma cells contains a substance capable of increasing vascular permeability. This substance is also found in cyst fluid from brain tumor patients. This vascular permeability factor (VPF) is probably at least partially responsible for the cerebral edema associated with brain tumors. Based on partial purification and biochemical characterization, VPF appears to be a tumor-specific cationic heparin-binding protein which is distinct from other substances known to elicit edema or regulate endothelial cell growth. Evidence to date suggests that the mechanism of action of VPF involves binding to endothelial cells through interaction with heparin-like moieties on the cell surface and subsequent influx of  $\text{Ca}^{+2}$ . We are continuing to study the effects of VPF on isolated endothelial cells to obtain further insight into mechanism of action of VPF. In addition, much of our effort is now being directed toward using our system to identify potential therapeutic agents, such as calcium channel blockers, which may inhibit the action of VPF and the resulting cerebral edema associated with brain tumors.

##### B. The Role of Insulin and Insulin-Like Growth Factors in Glioma Cells

Insulin and insulin-like growth factor (IGF) receptors have been detected in the human central nervous system. The role of IGFs in the nervous system is not well understood although IGF-1 may be involved in the growth of the peripheral nervous system, and IGF-2 may play a role in glial cell growth and maturation in the brain. The role of insulin and IGFs in glioma cells has not yet been investigated, although increased levels of IGF receptors have been found in certain other tumors, and the IGF-1 receptor shares sequence homology with the ROS oncogene. The number of epidermal growth factor receptors are also increased in some gliomas. We initiated studies to determine the nature of insulin and IGF receptors on human glioma cells derived from surgical specimens. Our results demonstrate (1) high levels of IGF-1 receptors on some gliomas; (2) the presence of receptors for IGF-1 and IGF-2, but not for insulin on cultured cells derived from human gliomas; and (3) IGF-1 receptors on cultured glioma cells are functional, as determined by the ability of IGF-1 to stimulate autophosphorylation of the receptor and DNA synthesis. In addition, high levels of high affinity IGF binding proteins (IGF BP) are produced by glioma cells and associated with some glioma surgical specimens. Certain characteristics of this IGF BP suggest it may be distinct from previously described IGF BPs and thus may have a function specific for glioma cells. These results suggest a role for IGF-1, its receptor, and its binding protein in the regulation of growth in some gliomas.

## V. Molecular Biology Unit

Iqbal Ali, Ph.D., Head

The Molecular Biology Unit of the Surgical Neurology Branch is planning to study the genetic abnormalities of various brain disorders, especially brain cancers. Extensive studies of the molecular origins of human cancer done over the past several years have implicated dominantly acting proto-oncogenes in various malignancies. These proto-oncogenes appear to have essential roles in normal cellular physiology and are most probably involved in regulatory functions. Several mechanisms, such as point mutations, rearrangement, amplification and/or elevated expression, are responsible for the conversion of these normal genes into oncogenes. Recently, yet another mechanism i.e. loss of gene function, is also believed to be one of the genetic events in the development of certain neoplasms.

Glial tumors, which account for the vast majority of primary tumors of the central nervous system, are exceptionally diverse in origin location, histology and biologic behavior. Malignant gliomas, with a whole spectrum of increasing anaplasia starting from well differentiated astrocytoma to anaplastic glioblastoma multiforme, offer an excellent model system to study molecular mechanisms that confer upon these cells an increased ability to grow, interact with the environment and eventually metastasize. A variety of genetic lesions may therefore be expected to contribute to the expression of neoplastic phenotype of gliomas. We propose to take the following initial approaches designed for understanding the genetic aberrations responsible for the expression of malignant phenotype in glial tumors, which probably evolve through a series of genetic lesions.

I - Activation of ras oncogenes by point mutations has been identified in about 20% of human malignancies and can occur as initiating events or as part of the evolution of neoplastic transformation. We plan to screen glial tumors for the presence of point mutations that have been mostly detected so far in three hot spots, codons 12 & 13 of the first exon and codon 61 of the second exon, of all three members of the ras gene family, Harvey, Kirsten, and N-ras. The tumor DNA around the region of the ras genes will first be amplified by polymerase chain reaction. The amplified DNA will then be screened for the presence of various point mutations either by radiolabeled allele-specific probes or by restriction fragment length polymorphism analysis (RFLP). This technique, because of its high sensitivity, will not only permit the analysis of point mutations of tumors of very small size, but will also detect preneoplastic cells (where point mutations of ras genes may function as initiating events) in surgical biopsies.

1A - Another example of proto-oncogene activation by point mutation is c-erbB2, which was detected in a chemically induced rat neuroglioblastoma. Amplification of c-erbB2 gene has been detected in human primary breast cancers. We will screen various grades of gliomas for possible amplification, overexpression, and/or presence of point mutations in the transmembrane domain of the c-erbB2 proto-oncogene.

II - One of the characteristic features of glioblastoma is vascular proliferation suggesting a response to angiogenic factors. A transgenic animal model system suggests that induction of angiogenesis is a second necessary step for tumor formation. A wide variety of factors that share structure characteristic of signal-transmitting molecules seem to have both growth promoting and angiogenic

activities. These include acidic and basic fibroblast growth factors (FGFs), FGF-5, int-2, hst-1, c-sea, and several members of transforming growth factors (TFGs). A systematic analysis of the genetic integrity and level of expression of these factors in various grades of astrocytomas and glioblastomas compared to normal brain and other normal tissues can be expected to provide important information on the role of angiogenesis.

III - The loss of heterozygosity of specific genes, as detected by restriction fragment length polymorphism (RFLP) analysis, suggests the presence of oncogenetic mutations that are unmasked by the loss of the wild type alleles. Recently, allelic deletions of genes on chromosome 10 has been reported in glioblastoma multiforme. It is worthwhile to carry out a systematic (RFLP) analysis of different grades of astrocytomas and glioblastomas using the VNTR (variable number of tandem repeats) probes that have a high probability of detecting losses of heterozygosity. These deletions identify the map positions of genes with possible roles in the developmental and regulatory processes and will be helpful in their cloning and characterization.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02739-03 SN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Laboratory Investigation of Central Nervous System Vascular Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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David Barba, M.D.

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Marston Linehan, M.D.

Surgical Branch, NCI

Berton Zbar, M.D.

Senior Investigator, NCI

## COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC, Experimental Therapeutics Branch, NINDS  
Surgery Branch, National Cancer Institute

## LAB/BRANCH

Surgical Neurology Branch, CNP

## SECTION

Clinical Neurosurgery Section

## INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

2.5

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical features, arteriographic findings, and treatment of 81 patients with spinal arteriovenous malformations demonstrated that there are distinguishing clinical features in patients with arteriovenous malformations of the spinal cord compared to those of patients with arteriovenous fistulas of the spinal dura. The findings indicate that spinal arteriovenous fistulas are acquired lesions, and not congenital, as was previously thought, and support arteriovenous malformations of the spinal cord as congenital lesions. The findings also indicate that AVMs of the spinal cord produce myelopathy as a result of high blood flow, but that patients with spinal dural AV fistulas develop myelopathy as a result of increased venous pressure in the spinal cord.

Magnetic resonance imaging permits demonstration of the presence and site of arteriovenous malformations of the spinal cord and therefore is a valuable and safe, non-invasive technique to investigate patients suspected of having spinal AVMs.

Foix-Alajouine syndrome was demonstrated to be due to venous congestion, and not venous thrombosis, and therefore, amenable to reversal by treatment of the spinal AVM. Spinal AVMs were shown to recanalize consistently after embolic occlusion.

Patients with Von Hippel-Lindau disease were investigated and the following were shown: investigation of the molecular biology of the hemangioblastomas of the central nervous system revealed a homozygous deletion of a portion of the short arm of the third chromosome, demonstrating that these tumors are probably caused by the absence of a tumor suppressing gene, as are familial retinoblastomas. MRI with gadolinium EDTA contrast enhancement was shown to be a sensitive technique of detection for small hemangioblastomas of the central nervous system. Excision of the tumors alone was shown to result in resolution of syringomyelia associated with spinal cord hemangioblastomas. Therefore the tumor-associated syrinx does not need separate treatment.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02673-05 SN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibodies Linked to Ricin for Use in Human Bone Marrow Transplantation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Richard J. Youle, Ph.D.

Principal Investigator, SNB, NINDS

Pat Johnson

Bio Laboratory Technician, SNB, NINDS

Virginia Johnson, Ph.D.

Senior Staff Fellow, SNB, NINDS

## COOPERATING UNITS (if any)

American Red Cross; Washington Children's Hospital; National Cancer Institute, Immunology Branch, DCBD.

## LAB/BRANCH

Surgical Neurology Branch, CNP

## SECTION

Biochemistry Section

## INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.5

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bone marrow transplantation is the treatment of choice for high risk leukemia, aplastic anemia and other immunodeficiency disorders. It is also being used for therapy of other radiation sensitive tumors such as neuroblastoma and for inherited enzyme deficiency disorders. One complication of graft-versus-host disease (GVHD) caused by mature T cells in the donor marrow recognizing histocompatibility differences between donor and host. Studies in animals and humans have shown that removal of mature T cells from the donor marrow while preserving the pluripotent stem cells prevent GVHD. Autologous grafts avoid this complication but have the problem of tumor cells circumventing therapy by remaining in the marrow graft.

Monoclonal antibodies linked to toxic proteins can specifically kill cells based on cell surface antigen differences. We have developed a panel of T cell selective toxins which kill up to 5 logs of T cells at concentrations non-toxic to human stem cells.

We have begun to investigate immunotoxins for depletion of tumor cells from autologous bone marrow grafts. Initial studies show that CRM 107 immunotoxins selectively kill human glioblastoma, medulloblastoma, breast carcinoma, lung carcinoma and leukemia cells at nanomolar concentrations or less. Ongoing experiments will determine the sensitivity of human multipotent stem cells to immunotoxins.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02674-05 SN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibody-Toxin Conjugates for Tumor Therapy In Vivo

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

|                         |                                    |
|-------------------------|------------------------------------|
| Richard J. Youle, Ph.D. | Principal Investigator, SNB, NINDS |
| Virginia Johnson, Ph.D. | Senior Staff Fellow, SNB, NINDS    |
| Debbie Wilson           | Bio Lab Technician, SNB, NINDS     |
| Jin Fu-Sheng, M.D.      | Special Volunteer, SNB, NINDS      |
| Susanna Rybak, Ph.D.    | Special Expert, SNB, NINDS         |
| Charles Riedel, M.D.    | Special Volunteer, SNB, NINDS      |
| Karin Muraszko, M.D.    | Senior Staff Fellow, SNB, NINDS    |

## COOPERATING UNITS (if any)

Biogen  
Cetus Corporation

## LAB/BRANCH

Surgical Neurology Branch, CNP

## SECTION

Biochemistry Section

## INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

6

OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues

(c) Neither



(a1) Minors



(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal antibodies selectively bind tumor cell differentiating antigens in vitro and in vivo. Natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells so we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells.

Two methods of coupling toxic proteins, like ricin to antibodies, have been used to kill antigen positive cells in vitro. Ricin has two subunits, the A subunit blocks protein synthesis when in the cytosol and the B subunit binds galactose groups on all cell surfaces but also facilitates the transport of ricin A chain to the cytosol. 1) Linkage of the ricin A chain to antibodies yields reagents with low non-target toxicity but target cell toxicity too slow for in vivo applications; 2) Linkage of intact ricin to antibodies results in very potent target cell toxicity but the non-target cell killing must be prevented by a ligand which blocks ricin B chain binding to cells. This has limited its application to in vitro situations where 100 mM lactose can block ricin binding.

We have succeeded in developing several new approaches to apply immunotoxins in vivo. 1) Cloning of toxins then altering their structure at the gene level to decrease non-target cell toxicity; 2) Intrathecal administration of immunotoxins for therapy of brain tumors that kill 2-5 logs of tumor cells in animal models; 3) Preparation of genetically engineered immunotoxins for clinical trials of human brain tumor patients; 4) Prevention of immune response against immunotoxin with anti-CD4 antibodies; 5) Construction of HIV infected cell immunotoxins and 6) Specific deletion of Purkinje cells in rats, guinea pigs and rhesus.

|   |                          |  |
|---|--------------------------|--|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                          | <b>PROJECT NUMBER</b><br>Z01 NS 02729-03    SN |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989   |                          |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Adrenal Medullary Autografts in Parkinsonian Patients   |                          |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br><div style="display: flex; justify-content: space-between;"> <div>Robert J. Plunkett, M.D.</div> <div>Principal Investigator, SNB</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Kris Bankiewicz, M.D.</div> <div>Visiting Associate, SNB</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div>Jeff Norton, M.D.</div> <div>Senior Staff Fellow, NCI</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div>Hetty DeVroom, R.N.</div> <div>Clinical Nurse, SNB, NINDS</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div>Robert Miletich, M.D., Ph.D.</div> <div>Senior Staff Fellow, NIS, NINDS</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div>Edward Oldfield, M.D.</div> <div>Chief, SNB</div> </div>  |                          |  |
| <b>COOPERATING UNITS</b> (if any)<br>Surgery Branch, National Cancer Institute<br>Neuroimaging Section, NINDS   |                          |  |
| <b>LAB/BRANCH</b><br>Surgical Neurology Branch, CNP   |                          |  |
| <b>SECTION</b><br>Clinical Neurosurgery Section, Central Nervous System Implantation Unit   |                          |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, National Institutes of Health, Bethesda, Maryland 20892   |                          |  |
| <b>TOTAL MAN-YEARS:</b> 1.5   | <b>PROFESSIONAL:</b> 1.5 | <b>OTHER:</b> 0.0                              |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>  |                          |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p>This study is designed to assess the efficacy of <u>adrenal medullary autografts</u> in patients with severe <u>Parkinson's disease</u>. The patients selected will have Stage IV disease, but still show some response to oral dopamine <u>replacement therapy</u>. They will undergo extensive testing pre-operatively including gait, posture, reaction time, speech, and neuropsychiatric assessment. Cavities will be created in the right caudate nucleus via a transcallosal approach, and a ventricular reservoir placed. Two weeks later one adrenal gland will be removed, and placed into the performed cavity. The preoperative testing will be repeated at regular intervals after <u>implantation</u>. CSF <u>biochemistry</u> and 6-fluorodopa <u>PET studies</u> will also be part of the testing carried out.</p> <p>One patient has been studied thus far. He has shown mild improvement with reduction of the severity of his on/off fluctuations. In addition, he has had a lessening of the dyskinesias which limit his tolerance for oral therapy. The CSF studies have revealed an elevation of enkephalin in the first few months compared to pre-operatively. Other patients are under evaluation for entry into the protocol.</p> |                          |  |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02781-02 SN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tissue Implantation in Parkinsonian Models

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert Plunkett, M.D. Principal Investigator, SNB

Kris Bankiewicz, M.D. - Visiting Fellow, SNB  
 Mark Luciano, M.D. - Medical Staff Fellow, SNB  
 Jay Morgan, M.D. - Medical Staff Fellow, SNB  
 Ian McCutcheon, M.D. - Medical Staff Fellow, SNB  
 Scott Ewing, M.D. - Visiting Medical Student, SNB

JG Sheng, M.D. - Visiting Fellow, SNB  
 Bernhard Zunkler, M.D. - Visiting Fellow, SNB  
 D. Bell, M.D. - Visiting Medical Student, SNB  
 Jin Wang, M.D. - Visiting Fellow, SNB

## COOPERATING UNITS (if any)

Meg Palmatier, Ph.D. - Staff Fellow, Clinical Neuroscience Branch; R. Gress, M.D., Scientist, NCI  
 RJ Weber, Ph.D., Scientist, NIDDK

## LAB/BRANCH

Surgical Neurology Branch, CNP

## SECTION

Clinical Neurosurgery Section, Central Nervous System Implant Unit

## INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland

## TOTAL MAN-YEARS:

6.5

## PROFESSIONAL:

6.5

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The behavioral, biochemical, and histological effects of tissue implants in rodent and primate models of parkinsonism are being studied. Fetal dopaminergic grafts have lead to almost complete functional recovery in the monkeys, with lesser improvement seen after non-dopaminergic fetal grafts, adrenal grafts, amnion grafts, or operative trauma alone. In all the animals studied thus far, there has been sprouting of tyrosine hydroxylase-positive fibers into the denervated caudate, which may account for part of the recovery seen (especially in animals where no dopaminergic tissue survives). We are trying to determine the cell-to-cell interaction which leads to new growth of fibers from an adult neuron. We are employing in vivo and in vitro methods to isolate the neurite-promoting factor(s), including cell suspension implants in hemiparkinsonian rats.

Another important aspect of the project is to examine technical questions associated with implantation. The use of stereotactic cell deposition has been carefully studied and a reliable method in rat and primate brain worked out. We are now using magnetic resonance images to guide stereotaxis in the monkeys. The immunological factors involved in allotransplantation are under study, including HLA expression and peripheral evidence of rejection occurring within the brain. PET and glucose utilization studies after implantation are being correlated with functional changes and receptor subtypes are being analyzed. The use of an in vivo microdialysis catheter has been developed to assess dopamine metabolism. Finally, we are studying dopaminergic and other tissues for potential clinical use, including human fetal mesencephalon, term human amnion, and leukocytes.

|  |                          |   |
|--|--------------------------|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                          | <b>PROJECT NUMBER</b><br><br>Z01 NS 02728-03 SN |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |                          |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Studies on the Heterogeneity of Drug Delivery During Intracarotid Chemotherapy   |                          |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br><div style="display: flex; justify-content: space-between;"> <div style="width: 40%;">Stephen Saris, M.D.</div> <div style="width: 60%;">Senior Staff Fellow, Principal Investigator, SNB</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 40%;">           Robert Lutz, Ph.D.<br/>           Ron Blasberg, M.D.<br/>           Donald Wright, M.D.<br/>           Edward H. Oldfield, M.D.         </div> <div style="width: 60%;">           Staff Fellow<br/>           Senior Investigator, Department of Nuclear Medicine<br/>           Medical Officer, SNB, NINDS<br/>           Chief, SNB, NINDS         </div> </div> |                          |   |
| <b>COOPERATING UNITS</b> (if any)<br>Department of Nuclear Medicine  |                          |   |
| <b>LAB/BRANCH</b><br>Surgical Neurology Branch, CNP  |                          |   |
| <b>SECTION</b><br>Clinical Neurosurgery Section  |                          |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, National Institutes of Health, Bethesda, Maryland 20892  |                          |   |
| <b>TOTAL MAN-YEARS:</b> 1.0  | <b>PROFESSIONAL:</b> 1.0 | <b>OTHER:</b> 0.0                               |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>  |                          |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br>Our objective is to study the physiology of intraarterial infusions in the carotid artery. A major problem with intracarotid infusions in patients with gliomas is focal injury to the eye and brain. Our hypothesis is that these problems are due to poor mixing of the infusate with blood such that certain eye and brain areas receive toxic amounts of drug that result in infarction of tissue. Other areas may receive suboptimal drug delivery resulting in treatment failure. We demonstrated that in animal models, and are now studying patients. We hope to eliminate this poor mixing with a specifically engineered infusion pump which injects in a pulsatile manner during the slow blood flow phase of diastole.                          |                          |   |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02708-04 SN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Permeability Factor Produced by Human Glioma Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marsha Merrill, Ph.D.

Principal Investigator, SNB, NINDS

Calvin Hawkins

Technician, SNB

Lou Rosa, M.D.

Senior Staff Fellow, SNB

Edward Oldfield, M.D.

Chief, SNB

COOPERATING UNITS (if any)

Dr. Milton Brightman, Laboratory of Neurobiology, NINDS

LAB/BRANCH

Surgical Neurology Branch, CNP

SECTION

Clinical Neurosurgery Section, Tumor Biology Unit

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cerebral edema associated with malignant brain tumors causes neurologic deficits and increased intracranial pressure, and contributes to the morbidity and mortality of the neoplasm. We have determined that the conditioned medium of glioblastoma-derived cell cultures contains a substance capable of increasing the vascular permeability in a bioassay which measures the induction of capillary permeability in normal skin. Partial purification and biochemical characterization of this vascular permeability factor (VPF) suggest that it is a heparin binding cationic polypeptide which is distinct from other known inducers of vascular permeability such as histamine, prostaglandins, leukotrienes, and plasminogen activator. VPF-containing conditioned medium increased calcium ion influx in cultured endothelial cells. The calcium channel blocker LiCl was able to inhibit VPF activity both in cultured endothelial cells and in guinea pig skin. Although the mechanism of action of VPF remains unknown, the ability of VPF-containing conditioned medium to alter calcium flux in cultured endothelial cells suggests that VPF exerts its effect through a direct action on endothelial cells.



|  |  |   |
|--|--|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |  | <b>PROJECT NUMBER</b><br><br>Z01 NS 02778-02 SN |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |  |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Adoptive Immunotherapy of Brain Tumors   |  |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;">Stephen Saris, M.D.</div> <div style="width: 65%;">Principal Investigator, SNB, NINDS</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 30%;">           Aytac Akbasak, M.D.<br/>           Ikejiri, Barbara<br/>           Edward Oldfield, MD.         </div> <div style="width: 65%;">           Special Volunteer, SNB, NINDS<br/>           Biologist, SNB, NINDS<br/>           Chief, SNB, NINDS         </div> </div>   |  |   |
| <b>COOPERATING UNITS</b> (if any)<br>Surgical Oncology Branch, NCI   |  |   |
| <b>LAB/BRANCH</b><br>Surgical Neurology Branch, CNP  |  |   |
| <b>SECTION</b><br>Clinical Neurosurgery Section  |  |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, National Institutes of Health, Bethesda, Maryland 20892  |  |   |
| <b>TOTAL MAN-YEARS:</b><br><div style="display: flex; justify-content: space-between;"> <span>1.0</span> <span>PROFESSIONAL: 1.0</span> <span>OTHER: 0.0</span> </div>   |  |   |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>  |  |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p>There are 10-15,000 malignant <u>brain tumors</u> diagnosed each year. The most common of these are <u>glioblastomas</u>. Numerous treatments involving surgery, chemotherapy, and radiation are of palliative benefit only. We study the adoptive <u>immunotherapy</u> of malignant <u>brain tumors</u> with <u>interleukin-2 (IL-2)</u>, <u>lymphokine-activated killer (LAK) cells</u>, and tumor infiltrating <u>lymphocytes (TILs)</u> in <u>animal models</u> and in <u>patients</u>. In the 9L gliosarcoma rat model, we studied the efficacy of parenteral IL-2 and its effect on the blood-brain barrier of normal and neoplastic tissue, and in the C3H mouse we studied the cytolytic activity of LAK against metastases in the brain. In patients with extracranial cancer, we examined the kinetics of IL-2 in the cerebrospinal fluid as compared to serum, and in patients with gliomas we investigated the cerebral toxicity of parenteral IL-2. We are currently completing our studies with IL-2 and LAK cells, and are expanding our efforts with TILs. In animals models of primary and metastatic brain tumors, we have raised TILs against tumors in the subcutaneous space; these cells will then be used to treat animals with intracerebral tumors and to study trafficking across the blood-brain barrier with <sup>111</sup>Indium labeled cells. In patients, we are raising TILs from brain tumors grown in the subcutaneous space. These have been expanded in vitro with IL-2, and injected parenterally into mice to study lymphocyte trafficking and efficacy against intracerebral tumors. In patients we have raised TILs from fresh specimens sent from the operating room. This creates the possibility of proceeding with Phase I clinical trials based on the final outcome of our laboratory efforts. Lastly, we are investigating the regulation of major histocompatibility antigens of brain tumors <u>in vitro</u> by interleukins, interferons, and tumor factor.</p> |  |   |

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|--|-------------------------|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |                         | <b>PROJECT NUMBER</b><br><br>Z01 NS 02697-05 SN |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |                         |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Protection of the Brain Against Injury by Ionizing Radiation with Pentobarbital  |                         |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br><div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Jeffrey J. Olson, M.D.</div> <div>Principal Investigator, SNB, NINDS</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Edward H. Oldfield, M.D.</div> <div>Chief, SNB, NINDS</div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div>Craig Shelley, M.D.</div> <div>Medical Staff Fellow, SNB</div> </div>   |                         |   |
| <b>COOPERATING UNITS</b> (if any)<br>National Cancer Institute, Radiation Oncology Branch  |                         |   |
| <b>LAB/BRANCH</b><br>Surgical Neurology Branch, CNP  |                         |   |
| <b>SECTION</b><br>Clinical Neurosurgery Section  |                         |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, National Institutes of Health, Bethesda, Maryland 20892  |                         |   |
| <b>TOTAL MAN-YEARS:</b> 2.   | <b>PROFESSIONAL:</b> 2. | <b>OTHER:</b> 0.0                               |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start; margin-top: 10px;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%; text-align: center;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%; text-align: center;"> <input type="checkbox"/> (c) Neither         </div> </div>   |                         |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p>The efficacy of <u>radiation therapy</u> in the treatment of <u>brain tumors</u> is limited by the toxicity of ionizing radiation to the surrounding normal tissue.</p> <p>In the rat model of cerebral radiation injury, pentobarbital has been shown to enhance overall survival in a dose dependent manner. The mechanism of this improvement may be related to the ability of this compound to enhance the binding of GABA to its receptor with subsequent opening of the chloride ion channel. When given in combination with pentobarbital, picrotoxin, an inhibitor of GABA induced synaptic transmission, and bicuculline, an inhibitor of GABA binding were able to block the protective effects of pentobarbital. These findings further support the suspected role of membrane stabilization in the protective effect seen with pentobarbital.</p> <p>The <u>rodent model</u> of radiation injury does not parallel that of human injury. A <u>primate model</u> was designed to better assess the role of pentobarbital in circumstances more applicable to the human situation. This ongoing study has thus far revealed the ability of pentobarbital to limit the toxicity of the radiation utilized. No lesions have been visualized in the animal irradiated with pentobarbital when analyzed by MRI. Neuroendocrinologic evaluation has revealed early dysfunction of thyroid stimulating hormone, luteinizing hormone, growth hormone, and prolactin responses to stimulatory testing in the animals <u>radiation</u> while anesthetized with ketamine. Significantly less abnormalities have occurred in the pentobarbital group. Finally, the overall survival in the animals radiated with pentobarbital is markedly better than their ketamine treated counterparts.</p> |                         |   |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02707-04 SN

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Insulin and Insulin-like Growth Factors in Glioma Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marsha Merrill, Ph.D.

Principal Investigator, SNB

Nancy Edwards

Biologist, SNB

Jeffrey Olson, M.D.

Senior Staff Fellow, SNB

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, CNP

SECTION

Clinical Neurosurgery Section, Tumor Biology Unit

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Insulin and insulin-like growth factors (IGF-1 and -2 or somatomedins) are anabolic effectors in many tissues and cultured cells including astrocytes and neurons. Receptors for insulin and IGFs are found throughout the human brain. Receptors for several growth factors are increased in tumor vs. normal tissue and this discrepancy may have therapeutic value in targeting toxic agents to tumor cells. To evaluate the potential role of IGFs in human CNS tumors, we examined the level of insulin and IGF receptors in tumors (astrocytomas and glioblastomas) and in normal brain. Although all surgical specimens contain receptors for all three growth factors, the highest values were observed with IGF-1 binding to glioma specimens. The IGF-1 receptor in tumor is the same size (118 kDa  $\gamma$ -subunit) as the receptor in normal brain, confirming the neural origin of the tumor cells expressing the IGF-1 receptor. Cultured cells derived from glioma specimens also express IGF-1 receptors, and many of these lines demonstrate a functional receptor as indicated by stimulation of DNA synthesis and receptor autophosphorylation in response to IGF-1. This demonstration of functional IGF-1 receptors in glioma cells suggests a role for this receptor in the regulation of glioma cell growth. In addition, high levels of high affinity IGF binding proteins (IGF BP) are produced by glioma cells. Certain characteristics of this IGF BP suggest it may be distinct from previously described IGF BPs, and thus may have a function specific for glioma cells.

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| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |  | <b>PROJECT NUMBER</b><br><br>Z01 NS 02454-09 SN             |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |  |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br>Studies of Human Pituitary Tumors  |  |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br><div style="display: flex; justify-content: space-between;"> <span>Edward Oldfield, M.D.</span> <span>Chief, SNB, Principal Investigator, NINDS</span> </div>  |  |   |
| <b>COOPERATING UNITS</b> (if any)<br>Developmental Endocrinology Branch, NINDS<br>Diagnostic Radiology, CC   |  |   |
| <b>LAB/BRANCH</b><br>Surgical Neurology Branch   |  |   |
| <b>SECTION</b><br>Clinical Neurosurgery Section, CNP   |  |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, National Institutes of Health, Bethesda, Maryland 20892  |  |   |
| <b>TOTAL MAN-YEARS:</b><br><div style="text-align: center;">0.3</div>  | <b>PROFESSIONAL:</b><br><div style="text-align: center;">0.3</div> | <b>OTHER:</b><br><div style="text-align: center;">0.0</div> |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>   |  |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p>           We continue to investigate venous samplings of the <u>pituitary venous drainage</u> to aid in the <u>diagnosis</u> and <u>treatment</u> of patients with <u>Cushing's syndrome</u>. Over 200 patients have now received bilateral simultaneous inferior petrosal sinus (IPS) sampling. The results indicate that 1) procedure can be performed successfully in all patients with Cushing's syndrome (successful sampling has been performed in over 99% of the patients in whom it has been attempted); 2) the procedure distinguishes patients with ectopic ACTH secretion from those with pituitary adenomas with greater than 99% accuracy; 3) if one adds CRF stimulation to the diagnostic procedure, a diagnostic accuracy of 100% has been achieved in over 150 patients with Cushing's syndrome; 4) IPS sampling successfully determines which side of the pituitary gland microadenomas reside in patients with Cushing's disease with 85% accuracy; and 5) unilateral inferior petrosal sinus sampling, which is commonly used clinically, is frequently misleading.         </p> <p> <u>Repeat transsphenoidal surgery</u> is successful in irradiating the hyper- cortisolism of Cushing's disease in about 70% of patients. This therapy for patients with Cushing's disease after previous pituitary surgery has not previously been examined.         </p> <p>           The CRF stimulation test and the dexamethasone suppression test are equally accurate in determining and distinguishing patients with Cushing's disease from those with ectopic ACTH secretion in Cushing's syndrome. Both have approximately 10-15% <u>diagnostic</u> inaccuracy, when used alone, in our experience.         </p> <p> <u>CT scanning</u> of the pituitary gland is normal in 70% of patients with Cushing's syndrome. <u>MRI scanning</u> with and without <u>gadolinium-EDTA</u> was used to evaluate patients with Cushing's disease preoperatively. This technique permitted identification of the adenoma in about 50% of those patients with surgically proven microadenomas who had negative CT scans. It was learned that the timing of the MRI after the administration of gadolinium EDTA was critical in the optimal use of the technique, as sequential imaging demonstrated scans taken less than 5 min. after injection of contrasts to be most sensitive.         </p> |  |   |

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02367-11

SN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological, Immunological &amp; Chemotherapeutic Studies of Human Brain Tumors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul Kornblith, M.D.      Former Chief, SNB

## COOPERATING UNITS (if any)

## LAB/BRANCH

SNB

## SECTION

Clinical Neurosurgery Section, CNP

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒

(a) Human subjects

☒

(b) Human tissues

☐

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated (10/88)



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02686-05 SN

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunotherapy of Brain Tumors by IL-2 and Activated Lymphocytes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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## LAB/BRANCH

Surgical Neurology Branch, CNP

## SECTION

Clinical Neurosurgery Section

## INSTITUTE AND LOCATION

NINDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated (10/88)







ANNUAL REPORT  
October 1, 1988 through September 30, 1989  
Clinical Neuroscience Branch  
National Institute of Neurological Disorders and Stroke

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## ANNUAL REPORT

October 1, 1988 through September 30, 1989

### Clinical Neuroscience Branch

Clinical Neurosciences Program, DIR

National Institute of Neurological Disorders and Stroke

Irwin J. Kopin, M.D., Chief

The Clinical Neuroscience Branch conducts research on the role of neurotransmitter substances in normal brain and in peripheral autonomic function, the abnormalities in neurotransmitter metabolism and receptor activation in neurological disorders, and the effects of drugs and other therapies on neurotransmitters and the neuronal systems regulating their release and actions. The Branch is divided into two Sections. The Section on Clinical Neuropharmacology conducts research on neurotransmitter function in humans, whereas the Section on Aminergic Mechanisms examines animals and animal models of human neurological disorders to study the role of neurotransmitter substances in pathogenesis and treatment of neurological diseases, with particular emphasis on biogenic amines.

The Branch has a Unit on Growth Factors and a Unit on Brain Imaging which provides additional support for studies related to the development or regeneration and the function *in vivo* of specific neuronal systems. Much of this work is performed in collaboration with investigators in the Surgical Neurology Branch.

### CLINICAL NEUROPHARMACOLOGY SECTION

The Clinical Neuropharmacology Section continues to develop biochemical and pharmacological methods for the assessment of neurotransmitter function and metabolism in man. One of the primary applications for these strategies is the investigation and treatment of patients with autonomic nervous system disorders. Two distinct disorders have been the main focus of these efforts. Autonomic failure may occur alone (pure autonomic failure, PAF) or in association with central nervous

system degeneration (multiple system atrophy, MSA). Investigation of patients with lesion(s) of the autonomic nervous system provides an opportunity to examine the interaction between the autonomic nervous system and other hormonal/peptide control systems.

Our investigations have focused primarily on the noradrenergic system because norepinephrine is the transmitter released by most post-ganglionic sympathetic neurons and also plays an important role in central nervous system pathways concerned with the regulation of blood pressure. High performance liquid chromatography, liquid scintillation spectrometry, and gas chromatography-mass spectroscopy are used to measure neurotransmitter and metabolite levels in various biological fluids. The activities of synthetic and degradative enzymes related to neurotransmitter metabolism are determined by radioenzymatic assay. Peptide and hormonal levels are analyzed by radioimmunoassay. These substances are measured under basal conditions and after a variety of stimuli have been applied to evaluate the responsivity of the particular system under investigation.

Previously, we found that patients with MSA have deficient ACTH and  $\beta$ -endorphin responses to insulin-induced hypoglycemia. Since ACTH can be released through a central cholinergic mechanism, we further examined the abnormality in release of the peptide by administering a cholinergic agonist, arecoline, after pretreatment with glycopyrrolate to block peripheral muscarinic effects of the drug. Although baseline ACTH levels were similar among normal subjects and patients with either MSA or PAF, neither patient group increased plasma ACTH levels in response to arecoline. Plasma norepinephrine (NE) levels did not significantly change following arecoline administration; however, plasma epinephrine (EPI) levels increased in normal subjects and patients with MSA. Since catecholamines may be required to release ACTH under various physiological conditions, the deficient ACTH response to arecoline in PAF could result from the lack of a normal plasma EPI increment. These findings are consistent with involvement limited to the peripheral autonomic nervous system. In contrast, the diminished ACTH response in MSA is most likely related to central nervous system lesion(s). Hypothalamic degeneration in MSA is attended by a reduction in choline acetyltransferase, a marker of cholinergic neuronal integrity. Involvement of the hypothalamo-pituitary

cholinergic pathway may underlie the abnormality of ACTH release in MSA. Of interest, patients with PAF and normal subjects did not experience significant central side effects of arecoline as observed in patients with MSA. Further investigation will be required to confirm the proposed explanation of these findings. We plan to measure the ACTH responses to arecoline in normal subjects treated with a ganglionic blocking drug and also in patients with autonomic failure during an infusion of epinephrine.

Dysfunction at various levels of the neuraxis can cause neurogenic orthostatic hypotension. In order to further characterize the deficits in sympathetic function and distinguish patients with orthostatic hypotension we examined the patterns of plasma levels of dopa, norepinephrine, dihydroxyphenylglycol and dihydroxyphenylacetic acid. Normal plasma catechol levels in MSA suggest that peripheral sympathetic neurons remain relatively intact; decreased levels of all four catechols in PAF are consistent with functional impairment of sympathetic nerve endings. Patients with deficiency of dopamine- $\beta$ -hydroxylase manifested increased levels of dopa and dihydroxyphenylacetic acid attended by markedly decreased levels of norepinephrine and dihydroxyphenylglycol. These unusual patients appear to have compensatory increases in sympathetic nerve activity in the absence of norepinephrine synthesis. Small groups of PAF and MSA patients exhibited low levels of the other catechols. These patients might have normal synthesis, but decreased release of norepinephrine. In general, patterns of plasma catechols distinguished different groups of patients with orthostatic hypotension. However, it will be necessary to investigate more patients to determine whether different subtypes or stages of these illnesses can be identified.

Measurement of the levels of neurotransmitters and their metabolites in CSF provides an index of central nervous system neurotransmitter metabolism. We previously showed that central noradrenergic, dopaminergic, and serotonergic systems are involved in MSA, but not PAF. Patients with MSA also have low levels of CSF acetylcholinesterase, consistent with central cholinergic dysfunction. In addition to its role as a hormone, somatostatin also appears to function as a neurotransmitter and neuromodulator. CSF levels of somatostatin are reduced in patients with MSA, but normal in those with PAF. There was not a correlation between low levels of

somatostatin in MSA patients and the low levels of monoamine metabolites or acetylcholinesterase. Thus, it appears that somatostatinergic dysfunction is not directly related to impairment of these other neurotransmitter systems.

Our studies of ganglionic receptor sensitivity to intravenously administered acetylcholine have been extended to include higher doses of the drug. In normal subjects there is a semilogarithmic relationship between plasma NE levels and the dose of acetylcholine. Patients with PAF did not exhibit any increase in plasma NE levels. The dose-response curve in MSA appears shifted to the right of normal, suggesting a down-regulation of receptor sensitivity. However, additional MSA patients must be studied using higher doses of acetylcholine to confirm this initial impression. The sensitivity and reproducibility of analytical methods for measuring plasma levels of acetylcholine do not appear to be adequate at the present time.

A detailed analysis of clinical and laboratory data collected during a longitudinal study of 87 patients with autonomic failure (24 PAF, 63 MSA) has been completed. Neurological examination was consistently normal in PAF; three MSA subgroups can be distinguished: (1) cerebellar, (2) parkinsonian, and (3) mixed. A large proportion of men in the cerebellar group (11/12) contributes to the overall male preponderance in MSA. There was no significant difference in the mean ages of onset for patients with PAF and MSA (47.4 and 51.6 years respectively). The most common presenting symptoms were lightheadedness (PAF) and genitourinary dysfunction (MSA). Most patients with MSA develop neurological symptoms about 4 years after the onset of autonomic symptoms. In approximately 25% of MSA cases, neurological symptoms precede autonomic dysfunction by 2 years. A low plasma NE level has diagnostic value, particularly if observed early in the course of the disease. Only 5 patients with PAF died, after an illness duration of 22 years. In striking contrast, the disorder lasted only 8.3 years in the 46 deceased MSA patients. Supine hypertension did not alter the duration of the illness. Differences in clinical features, natural history and prognosis support the distinction of PAF and MSA as separate disorders.

Psychosocial histories and pedigrees have been obtained on all autonomic failure patients. This observational approach has been employed to determine whether social, environmental, or familial factors contribute to the development and



progression of these degenerative disorders. Environmental and occupational exposures to a variety of toxic substances have been documented. In particular, there appears to be a higher incidence of organic chemical, heavy metal and radiation exposures in patients with MSA compared to a control population matched for age, sex and social scale. First-degree relatives of patients with MSA also reported a variety of autonomic and neurological symptoms more frequently than a control group. These preliminary findings are currently being analyzed in more depth through a collaborative effort with the Environmental Epidemiology Branch, National Cancer Institute.

We continue to use the combination of fluorocortisone and ibuprofen as our primary regimen for treating orthostatic hypotension. Minoxidil has been abandoned for this purpose although it has proven to be a useful drug for managing supine hypertension in some patients with autonomic failure. The multi-center trial of midodrine has been discontinued in lieu of an outpatient study which is being conducted in an effort to improve recruitment. No definite improvement in blood pressure was observed in those patients treated with midodrine at our institution. Our sympathetic neural prosthesis is being further refined under contract with the G.M.S. Engineering Corporation, Columbia, Maryland. Chronic intravenous administration of norepinephrine over two months did not cause significant toxicity in dogs. Current technology will not provide sufficiently frequent non-invasive blood pressure measurements to allow accurate control of blood pressure by a short-acting pressor drug. Thus, it will be necessary to use an implantable extravascular cuff to transmit signals to the micropressor. This device should be available for clinical testing within six months pending approval by the U.S. Food and Drug Administration.

A number of other studies are currently in progress:

1. Recent reports of antibodies against substantia nigra in the CSF of patients with Parkinson's disease have suggested that immunological factors may play a role in the pathogenesis of the disorder. In a collaborative study with the Department of Histology, University of Goteborg, Sweden, we have observed a large number of MSA patients who appear to have CSF antibodies that bind to the locus ceruleus; other brain regions did not exhibit labelled neurons. CSF from normal subjects

infrequently reacted with medial septum and substantia nigra, but never with locus ceruleus. Further investigations will attempt to elucidate the nature and specificity of this abnormality.

2. We have modified the radioimmunoassay for neuropeptide Y to measure its concentration in human plasma. This assay will be used to measure the peptide in response to various physiological and pharmacological stimuli in an effort to determine whether it is co-released with catecholamines in patients with autonomic failure.

3. A study of the cardiovascular and hormonal responses to guanfacine has been initiated. Growth hormone and vasopressin responses will be measured. This drug is similar to clonidine, but is more selective in terms of its actions at adrenergic receptors. Part of the study is designed as a therapeutic trial because clonidine has been used to successfully treat orthostatic hypotension in some patients with autonomic failure.

4. Sympathetic nervous system responses to hypercarbia are being assessed by measuring the plasma NE and blood pressure responses to re-breathing. This study is part of an effort to evaluate central mechanisms involved in sympathetic control.

5. Infusions of atrial natriuretic factor will be given to orthostatic hypotension patients and control subjects to confirm the suspected insensitivity to the peptide in patients with MSA.

6. Sleep studies are being carried out to define the physiological changes that contribute to sleep apnea and abnormal respiratory control in patients with MSA.

7. Further analysis of cerebral glucose metabolism measured by fluorodeoxyglucose PET scanning reveals that many MSA patients have a reduction in cortical metabolism. This is not surprising in view of the mild impairment of intellectual function that can occur in the later stages of the illness. Correlation with other clinical and imaging data is in progress.

8. A second PAF patient died during the past year, but unfortunately neuropathological studies were hampered by changes induced during her prolonged, terminal care.

The Clinical Neuropharmacology Section has continued the study of familial Alzheimer's disease (AD) as a major priority within the scope of its research efforts. Alzheimer's disease remains a major medical and social problem since it is the common cause of irreversible, chronic dementia. Clinical and therapeutic studies in AD are significantly limited by accuracy and timing of diagnosis. Even when the most stringent clinical diagnostic criteria are carefully employed, approximately 20% of the cases do not have AD at autopsy. Thus, the main justification for studying the more uncommon, autosomal dominant subgroup of familial cases lies in the accuracy of diagnosis that may be inferred through post-mortem examination of other affected family members. We have continued the two major directions of our AD research: (1) to investigate genetic linkage in order to identify the primary molecular event underlying AD, and (2) to define the clinical and biochemical progression of the disease through a longitudinal investigation of affected and at-risk subjects. This approach will provide clues for earlier, accurate diagnosis and more rational treatment strategies for AD.

Additional samples have been submitted to the Coriell Institute for Medical Research, Camden, New Jersey, for the establishment of skin fibroblast and peripheral blood lymphoblast cultures under an intra-agency agreement between the NINDS and NIA. Currently, we are working with 18 families that have the autosomal dominant pattern of inheritance; more than 250 family members (affected, at-risk, escapees, spouses) have been examined and biopsied. This is part of an international collaboration organized and coordinated by our Section. Families from the U.S., Canada, Italy, France, and Germany are participating in this effort. Identification of new branches of these families as well as continued follow-up with the families to verify clinical status of at-risk members increases their value for genetic linkage studies. The cultures continue to serve as a renewable source of DNA and cells for basic research in AD.

Although genetic linkage studies carried out by our collaborators continue to demonstrate linkage between AD and a region near the centromere of chromosome

21, it has not yet been possible to achieve a significant lod score with a single marker. Efforts have been primarily hampered by a lack of informative markers for this region in the large pedigrees under investigation. Recently developed markers are currently being tested for linkage. In addition, we are continuing to ascertain new pedigrees, expand the number of participating members of the current families, and update the clinical status to provide the maximum amount of accurate data for the linkage analysis. Attempts to search for living descendents of the large Canadian family who may not have emigrated from England have not yet been successful. Further efforts are in progress to determine whether any of the familial cases in the Northumberland region of England might be related to this family. In the U.S., contact has been established with the Virginia Commission on Aging to trace the origin of families with Alzheimer's disease in Northumberland County, Virginia. There are a number of families in this region with names that are commonly found in the large Canadian pedigree.

Longitudinal investigations have continued and psychological support and genetic counseling have been given. Sibship characteristics including birth order, maternal age, number of offspring, seasonality, associated diseases, education, occupation and longevity have been examined. The most striking finding was related to longevity of family members. In some sibships, adult non-affected siblings outlived their affected siblings by 16 years. In six adult sibships with four or more deaths, affected individuals outlived their siblings by an average of 7 years. The significance of these observations and possible relationship to factors such as immune mechanisms that might affect longevity remains to be determined through molecular genetic investigations. A variety of coping strategies were expressed by spouses of family members affected by Alzheimer's disease. Extreme fatigue, depression, anxiety and social withdrawal have been the main reactions to their total situation. Guilt about having children who might face the same fate as their spouse was also common. Finger print patterns have been collected on 12 affected subjects and 40 at-risk family members. A detailed analysis of dermatoglyphic patterns is in progress to determine whether the increased incidence of ulnar loops reported in sporadic Alzheimer's disease occurs in the dominantly inherited form of



the disease. Furthermore, it will be possible to examine these changes in first-degree at-risk members in these families.

CSF levels of monoamine metabolites, neurotransmitter related enzymes, and peptides have been measured in affected and at-risk members of these families. CSF levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), the major brain metabolite of norepinephrine, are corrected for the plasma contribution to give an index of central norepinephrine turnover. Homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) levels in CSF have been used to assess the dopaminergic and serotonergic systems respectively. Unfortunately, there is not a comparable index for cholinergic neuronal function. However, it is possible to measure acetylcholinesterase (AChE) in CSF which may give some indication of cholinergic neuronal integrity. Patients with dominantly inherited Alzheimer's disease have low CSF levels of corrected MHPG, AChE, and somatostatin suggesting that noradrenergic, cholinergic and somatostatinergic systems are involved. There is a subgroup of at-risk subjects who also manifest low CSF MHPG and somatostatin. Of interest is that these individuals also have a small but significant rating on the Blessed Dementia Scale. Two of the at-risk subjects in this group have developed dementia during the longitudinal evaluation. Thus, it appears that noradrenergic and somatostatinergic systems may be among the earliest neurochemical changes involved in this degenerative disorder. Dopamine and serotonin are not affected in dominantly inherited Alzheimer's disease.

Cerebral glucose metabolism is globally reduced in affected patients, but temporal and parietal regions are most severely compromised relative to other areas. Region of interest analysis in the at-risk subjects has not identified any definite changes that precede clinical expression of the disease. However, two at-risk subjects have developed dementia that is attended by a reduction in cerebral glucose metabolic rate in the temporo-parietal areas bilaterally. This longitudinal investigation will be continued until 50% of the at-risk subjects develop the disorder.

Neuropathological investigations have focused on the hippocampus in eight cases from three pedigrees. Very consistent patterns of pathological involvement have been observed. The highest density of neurofibrillary tangles and senile



plaques was present in CA1-2. Virtually no plaques were observed in the presubiculum. Senile plaque diameter was greatest in CA-4. Although there were no overall differences among families in the densities of plaques and tangles, statistical analysis revealed an uncommon type of senile plaque in two of the families. This type of plaque was characterized by a striking amyloid core, devoid of argentophilic neurites. The highest density of this unusual plaque was found in CA-4. Pathologic involvement appears to be homogeneous among these three large pedigrees; however, there is heterogeneity within families regarding the amount of amyloid deposited within specific regions of the hippocampus. A neuropathological investigation of twins affected by Alzheimer's disease is in progress.

Additional studies that have been continued or in progress include:

1. An investigation of blood pressure responses to norepinephrine and measurement of urinary catecholamine excretion patterns is being conducted since the locus ceruleus is affected in most cases of Alzheimer's disease. Preliminary analysis in a small number of affected subjects suggests that the pressor response may be altered.

2. Plasma ACTH responses to arecoline are being measured to assess central cholinergic function in affected and at-risk family members.

3. In collaboration with Dr. Jesse Siskin, University of Kentucky, fibroblast cultures are being used to study calcium channel function. Thus far no definite abnormalities have been consistently observed in cells derived from affected subjects.

## SECTION ON AMINERGIC MECHANISMS

Studies in this Section on biochemical evaluation of aminergic mechanisms have been in collaboration with investigators in the NHLBI for examination of peripheral autonomic function and in the Surgical Neurology Branch, NINDS, for evaluation of brain aminergic function.

The studies on the autonomic nervous system have focused on measurements of levels of norepinephrine, dopamine, 3,4-dihydroxyphenylalanine (DOPA) and their

metabolites in body fluids to assess sympathetic neuronal function. The results of these studies have shown that DOPA, the amino acid precursor of catecholamines formed from tyrosine in the brain, adrenal medulla, and sympathetic neurons enters blood and is the major source of extraneuronal dopamine. The amounts of DOPA in plasma reflect catecholamine synthesis in peripheral sympathetic nerves. Although DOPA is also the precursor of melanin, this source does not contribute significantly to plasma DOPA. The intraneuronal metabolite of norepinephrine, 3-4-dihydroxyphenylglycol (DHPG), is an index of sympathetic neuronal integrity which in combination with plasma norepinephrine levels provides information about sympathetic neuronal function. DOPA is formed mainly from NE which "leaks" into the cytoplasm from storage vesicles, but some is formed from NE which is recaptured after release into the synapse.

The venous-arterial differences in radiolabelled and unlabelled NE and blood flow provide a means of assessing the rate of overflow of NE from the sympathetic nerves in a tissue. Overflow of this amine generally correlates with sympathetic nerve activity, as does release of DOPA and DHPG into the circulation. DHPG clearance, however, may be altered by changes in the distribution of blood flow so that its plasma levels may be elevated even when net release is diminished.

Simultaneous estimation of urinary excretion rates on plasma levels of HVA (the major metabolite of dopamine) and of MHPG and VMA (the major metabolites of NE) before and during administration of debrisoquin are used to estimate the rate of brain dopamine metabolism. This method, which was verified using animals treated with MPTP to eliminate brain dopaminergic neurons, is now being used to study dopamine metabolism in humans.

$^{18}\text{F}$ -labelled dopamine has been shown to be a useful compound for imaging sympathetic nerves and their activity *in vivo* in experimental animals. In dogs, denervation of the salivary gland by removal of one superior cervical ganglion results in loss of uptake and retention of the  $^{18}\text{F}$ -label. Furthermore, the  $^{18}\text{F}$  accumulated in the heart declines more slowly when sympathetic nerve traffic is diminished. Thus,  $^{18}\text{F}$ -DA appears to be converted to  $^{18}\text{F}$ -NE in sympathetic nerves and, as we had previously shown in rats, can be used to reflect NE turnover.

Investigators in the unit on growth factors have been examining the regulation of synthesis and expression of neurotrophic agents and neuropeptides. Three different animal models of degenerative neurological disorders have been used to search for growth factors which promote neurite outgrowth and catecholamine uptake *in vitro* by neurones from chick dorsal root ganglia, rat superior cervical ganglia, or rat CNS neurons in culture. These tissues are also examined for m-RNA which is used to prepare cDNA libraries which are probed for NGF-like peptides.

C6 glioma cell line and primary cultures of astrocytes are being studied as models to understand the molecular mechanisms for regulating the synthesis of NGF systems. The C6 cells contain NGF mRNA and NGF protein, which is secreted into the medium.  $\beta$ -Adrenergic agonists stimulate synthesis by increasing transcription of the NGF gene. NGF mRNA has been detected in primary cultures of rat cortical, cerebellar and striatal astrocytes, and Schwann cells. NGF synthesis can be increased in astrocytes by both forskolin and isoproterenol, supporting the idea that in these cells also, cyclic AMP regulates transcription of the NGF gene. VIP (vasoactive intestinal peptide) can also stimulate NGF synthesis in astrocytes. This finding is of particular interest since in spinal cord cultures, VIP acts on astrocytes to release neurotrophic factors. Astrocytes can express certain neuropeptide genes, with both gene - and brain region specificity. Astrocytes from cortex, cerebellum and striatum all express the proenkephalin (PE) gene. The cells synthesize and process the precursor to the free enkephalin peptides. The pattern of processing appears to vary with the brain region. Neuropeptide expression is also developmentally regulated. Enkephalins or somatostatin may act as astrocyte-derived trophic factors for neurons early in development. The developmental time course of expression of proenkephalin (PE) mRNA in striatum is biphasic. The first peak occurs at a time when no synapses have yet formed, but when astrocyte expression is maximal. The second peak occurs at the time of active synapse formation. Analyses are currently underway to determine whether *in vivo* PE and SS gene expression occurs in astrocytes early in development.

Measurements of precursor mRNA, of precursor proteins, and of the biologically active peptides can determine whether a drug affects a neuropeptide by changing the transcription of the gene, the rate of translation to or processing of the precursor, or the utilization of the peptide itself. Drugs, such as morphine, can affect

peptide levels independent of an action on the rate of biosynthesis. These studies are complemented by the use of *in situ* hybridization to analyze changes in neuropeptide/neurotransmitter mRNAs at the single cell level.

The studies in the Unit on Neural Imaging have examined local cerebral glucose utilization and dopamine receptor distribution in MPTP parkinsonian primates and the changes in local brain metabolism during reward stimulation. In MPTP-hemiparkinsonian animals there are increases in metabolism in the striatum external globus pallidus and decreased metabolism in the subthalamic nucleus. Apomorphine causes generalized reduction in brain glucose utilization, depressing further the decrease in metabolism in the subthalamic nucleus and reducing the increase in metabolism in the striatum and external globus pallidus.

A new method has been developed to administer 2-<sup>14</sup>C deoxyglucose to freely moving rats. Apomorphine lowers brain glucose utilization in brains in intact rats and in animals in which 6OH DA had been used to destroy the substantia nigra.

Methamphetamine increases local cerebral glucose utilization (LCGU) in both mesocortical limbic dopaminergic and in the extrapyramidal motor systems. Long term (2 weeks) administration of this drug depresses LCGU in the striatum, extrapyramidal system and in some limbic areas.

Rats treated with cocaine have significant increases in expression of a specific neural protein, FOS, which is believed to influence gene expression. This effect appears to be mediated by dopamine D<sub>1</sub> receptors. Similarities of effects of drug abuse (cocaine and morphine) and rewarding brain stimulation on LCGU in the nucleus accumbens and olfactory tubercle have been described and suggest that the mechanism of addition may be related to "reward" areas of brain.



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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>  |                        | PROJECT NUMBER<br>Z01 NS 02716-04<br>CNB |
| PERIOD COVERED<br>October 1, 1988 through September 30, 1989   |                        |  |
| TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)<br>Neurotoxins and Animal Models of Neurological Diseases  |                        |  |
| PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>PI: Irwin J. Kopin, M.D., Chief, CNB, Director, DIR, NINDS<br>Others (CNB, NINDS):<br>Virginia Weise, Chemist; Alessandro Zuddas, M.D., Visiting Fellow; Linda Porrino, Ph.D., Research Psychologist; Mark Duncan, Ph.D., Visiting Fellow; Ann M. Marini, M.D., Sr. Staff Fellow; Margaret Palmatier, Ph.D., Staff Fellow; Judith Harvey-White, B.S., Technician.<br>Sanford P. Markey, Ph.D., Pharmacologist, LCS, NIMH<br>Krzysztof S. Bankiewicz, M.D., Visiting Fellow, SNB   |                        |  |
| COOPERATING UNITS (if any)<br>Surgical Neurology Branch, NINDS<br>Laboratory of Clinical Science, NIMH   |                        |  |
| LAB/BRANCH<br>Clinical Neuroscience Branch, CNP  |                        |  |
| SECTION<br>Aminergic Mechanisms  |                        |  |
| INSTITUTE AND LOCATION<br>NINDS, NIH, Bethesda, MD 20892   |                        |  |
| TOTAL MAN-YEARS:      3.2  | PROFESSIONAL:      2.2 | OTHER:      1.0                          |
| CHECK APPROPRIATE BOX(ES)<br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>  |                        |  |
| SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)<br><p>             The objectives are to identify and use <u>toxic substances</u> which target specific <u>neuronal systems</u> to produce <u>animal models of neurological disease</u> and to explore the <u>mechanisms of action</u> of such <u>toxins</u> as a means of examining: (1) The biochemical basis of neuron vulnerability to damage; (2) The role of such vulnerability in the pathogenesis of neurological disease; and (3) Approaches to new therapies for diseases of the nervous system. At present, 1-methyl-4-phenyl-1-2,3,6-tetrahydropyridine (MPTP) is the toxin most intensively studied, but other toxins such as beta-methylaminoalmine (BMAA) or 2-amino-3-methylamino-propanoic acid are being examined as well. MPTP is injected into mice at various doses, after various treatments, and the effects on movements observed. In collaborative studies with investigators in SNB, monkeys are treated with MPTP either intravenously or via the internal carotid artery. These animals are used to evaluate biochemical changes in body fluids (homovanillic acid, MHPG), effects on motor activity of DOPA and dopamine receptor agonists, alterations of dopamine neurons or receptors in various areas of brain as described for mice and by PET imaging with 18F-DOPA, or 2 DG radioautography. Implants of fetal tissue from the substantia nigra region are being used in attempts to reverse dopaminergic deficits in the basal ganglia of these animals. Compounds implicated as neurotoxins are synthesized with isotopically labelled components so that the occurrence, metabolism and distribution in the body of experimental animals can be examined. BMAA has been successfully synthesized using deuterium labelled methylamine and the isotopically labelled compound used to develop a gas chromatography-mass spectrometry method for assay of the suspected toxin in food products, body fluids and tissues. The BMAA content of cycad seeds of various types from Guam and other tropical areas has been determined and the process developed for preparation of the seeds for human consumption shown to remove almost all the toxin. During this process, however, in some preparations of the flour, zinc appears to have accumulated in sufficiently high concentrations to reach toxic levels. The role of zinc in the neurological disorder is now being examined.           </p> |                        |  |



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| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |   | <b>PROJECT NUMBER</b><br><b>Z01 NS02717-04</b><br><b>CNB</b>                 |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |   |  |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br><b>Biochemical Evaluation of Aminergic Function During Responses to Stress and in Disease States</b>   |   |  |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)<br>PI: Irwin J. Kopin, M.D., Chief, CNB, DIR, NINDS; Director, DIR, NINDS<br>Others (CNB, NINDS): Graeme Eisenhofer, Ph.D., Visiting Fellow; Katalin Szemerédi, Ph.D., Visiting Fellow; Anna Starosta, M.D., Visiting Fellow; Moshe Garty, M.D., Visiting Scientist; Ronald Polinsky, M.D., Chief, Clinical Neuropharmacology Section; Virginia K. Weise, Chemist; Judith Harvey-White, Technician<br>Others: David Goldstein, M.D., Ph.D., Medical Officer, HEB, NHLBI; Robin Stull, Technician, HEB, NHLBI  |   |  |
| <b>COOPERATING UNITS</b> (if any)<br>Hypertension-Endocrine Branch, NHLBI  |   |  |
| <b>LAB BRANCH</b><br>Clinical Neuroscience Branch, CNP   |   |  |
| <b>SECTION</b><br>Aminergic Mechanisms   |   |  |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, Bethesda, MD 20892  |   |  |
| <b>TOTAL MAN-YEARS:</b><br><div style="text-align: right; margin-right: 20px;">3.4</div>   | <b>PROFESSIONAL:</b><br><div style="text-align: right; margin-right: 20px;">3.4</div> | <b>OTHER:</b><br><div style="text-align: right; margin-right: 20px;">0</div> |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects<br/> <input type="checkbox"/> (a1) Minors<br/> <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>   |   |  |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><p>The main objectives are to develop methods for <u>biochemical evaluation of catecholaminergic neuronal function</u> and apply these methods to examining the role of these <u>neurons</u> during stress and in disease states. By the use of radioactively labelled amines and/or measurements of levels of DOPA, dopamine, norepinephrine, epinephrine and their metabolites in body fluids (CSF, plasma, urine), rates of amine formation and metabolism are estimated. Relative changes in rates of formation of the amines and selected metabolites provide indices of brain dopaminergic or noradrenergic activity or peripheral sympathoadrenal medullary function. Use of 18F-labelled dopamine is being developed for PET scanning of peripheral sympathetic activity <i>in vivo</i>. The rate of release of norepinephrine from sympathetic nerve terminals is reflected partially by spillover of the released catecholamine into the plasma, but only a small and somewhat variable portion reaches the circulation. At least two vesicular storage sites for norepinephrine have been demonstrated in the sympathetic nerves of the isolated rat vas deferens. Plasma DOPA level is an index of catecholamine formation since plasma DOPA is not derived from melanin-producing cells. The release of norepinephrine into the synapse is dependent upon outflow of impulses from the spinal cord, but is regulated also by negative feedback through presynaptic alpha-2 adrenoceptor stimulation by the released catecholamine. Fluorinated derivatives of DOPA and dopamine have been shown to be metabolized similarly to their parent compounds. Pharmacological inhibition with Debrisoquin of formation of catechols in peripheral tissues is attended by decreases in both dopamine and norepinephrine metabolites. The decline in excretion (or plasma levels) of these metabolites is not proportionately equal because HVA production in the brain is unaffected by debrisoquin. Based on our previous demonstration that HVA excretion attributable to brain dopamine is diminished by about 75% in MPTP treated monkeys, a method is now being used to estimate the rate of HVA production in brains of humans at different ages or with neurological disorders.</p> |   |  |

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|--|---|---|
| <b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b><br><b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>   |   | <b>PROJECT NUMBER</b><br><br>ZO1 NS 02630-06 CNB  |
| <b>PERIOD COVERED</b><br>October 1, 1988 through September 30, 1989  |   |   |
| <b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.)<br><b>CLINICAL, GENETIC, AND BIOCHEMICAL STUDIES OF FAMILIAL ALZHEIMER'S DISEASE</b>  |   |   |
| <b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)   |   |   |
| P.I.:  | R. J. POLINSKY, M.D.  | CHIEF<br><br>CNS, CNB, NINDS  |
| OTHERS:  | L.E. NEE, M.S.W.<br>S.M. BASER, M.D.<br>A.M. MARINI, M.D.<br>G. DI CHIRO, M.D.<br>J. GRAFMAN, PH.D. | SOCIAL SCIENCE ANALYST<br>MEDICAL STAFF FELLOW<br>SENIOR STAFF FELLOW<br>CHIEF<br>PSYCHOLOGIST<br><br>OCD, CNP, NINDS<br>CNS, CNB, NINDS<br>CNS, CNB, NINDS<br>NIS, OCD, NINDS<br>CNU, MNB, NINDS |
| <b>COOPERATING UNITS</b> (if any)<br><br>LAB OF HISTOPATH., LA SALPETRIERE (J. FONCINI); GENETICS UNIT, DEPT. OF NEUROLOGY, MASS. GEN. HOSP. (J. GUSELLA, P. HYSLOP); NEUROPATH LAB., JOHNS HOPKINS HOSP. (D. PRICE, R. STRUBLE); SMID-SUD, ITALY (A. BRUNI); KLINIK BAVARIA (P. FROMMELT); NEWCASTLE, ENGLAND (D. KAYE); UNIV. OF KY (J. SISKEN)  |   |   |
| <b>LAB BRANCH</b><br>CLINICAL NEUROSCIENCE BRANCH, CNP, DIR, NINDS   |   |   |
| <b>SECTION</b><br>CLINICAL NEUROPHARMACOLOGY SECTION   |   |   |
| <b>INSTITUTE AND LOCATION</b><br>NINDS, NIH, BETHESDA, MD 20892  |   |   |
| TOTAL MAN-YEARS:   | 4.0   | PROFESSIONAL: 3.0<br><br>OTHER: 1.0   |
| <b>CHECK APPROPRIATE BOX(ES)</b><br><input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither<br><input checked="" type="checkbox"/> (a1) Minors<br><input checked="" type="checkbox"/> (a2) Interviews  |   |   |
| <b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)<br><br><p> <u>Alzheimer's disease</u> (AD) is the most common cause of irreversible, chronic <u>dementia</u>. Although AD may be familial in only one third of all cases, the main justification for studying autosomal dominant cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members. More than 250 members of 18 pedigrees with an autosomal dominant form of AD have had <u>skin fibroblast</u> and <u>peripheral blood lymphoblast</u> cultures established. These cultures serve as a renewable source of DNA and cell lines for genetic linkage, viability, and biochemical studies. Recombinant DNA technology has been applied to perform genetic linkage studies in these families with inherited AD. Additional families have provided further confirmation of our earlier finding of a region of <u>chromosome 21</u> that is linked to AD; however, a single marker has not been identified. CSF levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), corrected for the plasma contribution, as well as acetylcholinesterase and somatostatin are low in patients with dominantly inherited AD, consistent with involvement in the noradrenergic, cholinergic and somatostatinergic systems. Indices of central dopamine and serotonin turnover are normal. A sub-group of first-degree at-risk relatives of patients with dominantly inherited AD also manifest low levels of MHPG and somatostatin in the CSF. These individuals also have a positive rating on the Blessed Dementia Scale although they have no clinical symptoms. Noradrenergic and somatostatinergic neurons may be among the earliest neurochemical systems affected by the degenerative process in AD. Detailed region of interest analysis of cerebral glucose metabolism has not revealed any definite changes that precede clinical expression of the disease. A single genetic marker for AD will facilitate analysis of any neurochemical and metabolic changes in at-risk subjects since they can be grouped according to whether they carry the AD gene. Neuropathological involvement appears to be homogeneous among three large pedigrees with multiple autopsies available for investigation. There is, however, heterogeneity within families regarding the amount of amyloid deposited in hippocampal regions.         </p> |   |   |





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS 02752-03  
CNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Synthesis and Expression of Neurotrophic Agents and Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Joan P. Schwartz, Ph.D., Research Chemist, CNB, NINDS

## Others:

Hisaharu Shinoda, M.D., Visiting Fellow, CNB, NINDS

Margaret Palmatier, Ph.D., Staff Fellow, CNB, NINDS

Cristina Cosi, Ph.D., Visiting Fellow, CNB, NINDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Clinical Neuroscience Branch, CNP

## SECTION

Section on Aminergic Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.6

## PROFESSIONAL:

2.6

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence suggests that parallel biochemical and regulatory processes occur during normal development and following various forms of CNS injury. Among these areas of particular interest are (1) the identification of CNS neurotrophic factors and (2) the analysis of the regulation of neuropeptide gene expression during development and in response to injury. Studies are underway to identify trophic factors produced in specific model systems, since recent evidence suggests that a family of nerve growth factors exists, each specific for certain populations of neurons. An NGF-like factor increases in the cerebellum of the pcd mutant mouse as the Purkinje cells die out and astrocytes proliferate. The mRNA for this factor appears to hybridize with mouse  $\beta$ -NGF cDNA and is increased in pcd cerebellum. Screening of cDNA libraries is currently in progress, in order to clone the factor. In another injury paradigm, multiple cortical lesions are made in rat brain: one week later, RNA is prepared from various brain regions to be analyzed for any change in NGF-like mRNAs relative to unlesioned brain. MPTP-lesioned animals (both mice and monkeys) represent a Parkinson-like model in which changes in NGF-like molecules are being examined. Since astrocytes can synthesize NGF, primary cultures of astrocytes are being used to determine factors which regulate NGF gene transcription as well as to assess production of other potential trophic factors.

At the same time, these injury models can be evaluated for changes in neuropeptide and/or neurotransmitter synthesis occurring in response to the lesions. One can derive an estimate of peptide turnover by combining measurements of the precursor mRNA, the precursor, and the peptide. Our studies have demonstrated that peptides are differentially regulated by such chronic drug treatments as reserpine, haloperidol, 6-hydroxydopamine or 5,7-dihydroxytryptamine. Work is in progress to determine the effects of CNS injury and recovery, including MPTP treatment, on various neuropeptides as well as such neurotransmitter synthetic enzymes as tyrosine hydroxylase and GAD, and the dopamine D2 receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 NS 02760-02  
CNB

PERIOD COVERED

October 1, 1988 through September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolic Mapping of the Brain During Rewarding Brain Stimulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Linda Porrino, Ph.D., Research Psychologist, CNB, NINDS

Francesco Pontieri, M.D., Visiting Fellow, NIMH

Stephanie Young, Howard Hughes Research Scholar, NIMH

COOPERATING UNITS (if any)

Behavioral Pharmacology Laboratory, Boston University School of Medicine (Conan Kornetsky, Ph.D.)  
Department of Pharmacology, Univ. of Chicago, (Lewis Seiden, Ph.D., & Mark Kleven, Ph.D.)

LAB/BRANCH

Clinical Neuroscience Branch, CNP

SECTION

Section on Aminergic Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

.30

PROFESSIONAL:

.20

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence suggests a critical involvement of the dopaminergic mesocorticolimbic system in positive reinforcement. A frequently employed model used to study reward behavior is electrical brain stimulation reward (BSR) in which animals perform a task in order to receive electrical stimulation to a discrete brain area. The deoxyglucose method is being used to study alterations in brain metabolic activity during BSR and following the administration of drugs with euphorogenic properties. The deoxyglucose method has been used to identify the sites of action of the reward-enhancing effects of cocaine and morphine in BSR paradigms. These data demonstrate that cocaine and morphine may exert their euphorogenic effects in this paradigm through actions at the same site, the olfactory tubercle. The long term consequences of the administration of drugs classified as abused substances have also been studied with the deoxyglucose method. In these studies, reductions in glucose utilization in the dorsal and ventral striatum were evident after chronic methamphetamine treatment. Immunocytochemical studies were carried out to measure expression of c-Fos protein which is thought to act as a genetic transactivator regulating gene expression. Increased presence of Fos was observed in the striatum of rats treated with cocaine, suggesting a possible mechanism for the long term effects of cocaine.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02761-02  
CNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolic Mapping of a Primate Model of Parkinsonism

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Linda Porrino, Ph.D., Research Psychologist, CNB, NINDS

Others: Irwin Kopin, M.D., Chief, CNB; Director, DIR, NINDS  
Krzysztof Bankiewicz, M.D., Visiting Fellow, SNB, NINDS  
John Viola, Howard Hughes Medical Fellow, NIMH  
Francesco Pontieri, M.D., Visiting Fellow, NIMH

## COOPERATING UNITS (if any)

Surgical Neurology Branch, NINDS  
Laboratory of Cerebral Metabolism, NIMH

## LAB/BRANCH

Clinical Neuroscience Branch, CNP

## SECTION

Section on Aminergic Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

.8

## PROFESSIONAL:

.4

## OTHER:

.4

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Intracarotid injection of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produces unilateral destruction of the dopaminergic cells of the ipsilateral substantia nigra pars compacta in primates. The 2-[<sup>14</sup>C]deoxyglucose method has been used in these experiments to map the neural circuits involved in the treatment of these hemiparkinsonian monkeys with dopaminergic agonist drugs and with surgical implants. Metabolic studies in hemiparkinsonian monkeys, later replicated in rats, demonstrated non-specific depressant effects of the dopaminergic agonist, apomorphine, on glucose metabolism in both MPTP-treated and untreated hemispheres. The deoxyglucose method was also used to assess functional recovery in hemiparkinsonian monkeys following surgical intervention. The pattern of glucose utilization seen in monkey with only surgical cavitation of the caudate nucleus in which partial behavioral recovery was observed was similar in part to the pattern observed in animals treated with L-DOPA.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02762-02  
CNB

## PERIOD COVERED

October 1, 1988 through September 30, 1989

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quantitative Autoradiographic Determination of Dopamine Receptor Distribution in Primates

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Linda Porrino, Ph.D., Research Psychologist, CNB, NINDS

Others: Francesco Pontieri, Visiting Fellow, NIMH

John Viola, Howard Hughes Medical Fellow, NIMH

Krzysztof Bankiewicz, M.D., Visiting Fellow, SNB, NINDS

Irwin J. Kopin, M.D., Branch Chief, CNB; Director DIR, NINDS

## COOPERATING UNITS (if any)

Laboratory of Cerebral Metabolism, NIMH

Surgical Neurology Branch, NINDS

## LAB/BRANCH

Clinical Neuroscience Branch, CNP

## SECTION

Section on Aminergic Mechanisms

## INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

.9

## PROFESSIONAL:

.4

## OTHER:

.5

## CHECK APPROPRIATE BOX(ES)

☐

(a) Human subjects

☐

(b) Human tissues

☒

(c) Neither

☐

(a1) Minors

☐

(a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The primate model of hemiparkinsonism produced by the intracarotid infusion of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), allows the study of neurochemical changes associated with lesions of the substantia nigra pars compacta. Quantitative autoradiography after *in vitro* radioligand binding with [<sup>3</sup>H]Sch23390 (D<sub>1</sub> antagonist) and [<sup>3</sup>H] sulpiride (D<sub>2</sub> antagonist) were used to determine the distribution of receptor sites in MPTP-induced hemiparkinsonian monkeys. Alterations in the distribution of both D<sub>1</sub> and D<sub>2</sub> receptor binding sites in the caudate and putamen on the side of the lesion were associated with significant increases in B<sub>max</sub> as compared to the untreated hemisphere, but not with changes in K<sub>d</sub>. MPTP lesions then, not only cause alterations in D<sub>2</sub> receptor population density, but in D<sub>1</sub> receptors as well.



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